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THE TRANSFORMATION OF PHOSPHORUS DURING THE DECOMPOSITION OF PLANT MATERIALS[1]

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Organic materials that are likely to reach the soil generally contain considerable phosphorus, ranging from 0.1 to 0.5 per cent (1, 2, 4, 5, 7, 14, 16, 17, 18, 19, 21, 23). Much of the phosphorus in the materials is in organic combinations which are not readily available to plants; the release of this great quantity of phosphorus in the form of readily available phosphate depends upon the decomposition of the organic compounds by microorganisms. From the point of view of phosphorus conservation, farm manures, green manures, and composts of various organic residues should not be considered merely as a source of nitrogen. The idea that phosphorus is completely mineralized during the decomposition of organic matter and can all be absorbed by the plant is too generally accepted.

Despite the many interesting studies on the biological solution of insoluble mineral phosphates, on the assimilation of phosphorus by soil fungi and bacteria, on the presence and abundance of organic phosphorus compounds in the soil, and on the microbial reduction of phosphate, the biological transformation of phosphorus, particularly that phase concerned with the formation and mineralization of organic phosphorus, during the decomposition of plant residues and green manures and during the composting has been the subject of but little investigation.

Working with different kinds of stable manure as sources of phosphoric acid, Egorov (9) noted a reduction in the amount of inorganic phosphates in the unsterilized fermenting manure. He was led to suggest the biological transformation of inorganic phosphorus to organic phosphorus during the decomposition of the organic matter. Duschechkin (8) pointed out that, in addition to physicochemical absorption of phosphorus in the soil, there is also biological absorption or fixation, which increases with an increase in the amount of organic matter (starch) added and also with the length of time. Doryland (5) pointed out that molds are active in the assimilation of plant-food constituents during the first stage of decomposition of crop residues when there is a high energy nutrient ratio; the molds may later play just as important a part as

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do bacteria in liberating plant-food constituents when the ratio is low. Tottingham and Hoffman (22) showed that during a period of fermentation of 4 to 6 months, the water-soluble phosphoric acid in manure and in a manure-floats mixture decreased. The reduction was thought to be due to the formation of nuclein compounds by the bacterial cells. The phosphorus thus made unavailable was eventually released in an available form as a result of the action of bacteria on the dead microbial cells after most of the readily decomposable material had been used up. Hutchinson (13), Heck (11), and Tam and Magistad (20) obtained similar results and held the same point of view. The recent data presented by Lockett (15) show an increase in the amount of inorganic phosphorus during the process of decomposition of rye and oat plants. A reversal was obtained after decomposition of the materials for 80 days.

It seemed desirable, therefore, to determine quantitatively what happens to the phosphorus originally present in, or added to, the organic materials when these undergo microbial decomposition. Such a study might be expected to provide information regarding the biological nature of the transformation of phosphorus during the decomposition of the organic matter and the availability of the phosphorus undergoing transformation. The results reported in this paper deal with the transformation of phosphorus during the decomposition of complex organic materials; the transformation of phosphorus during the decomposition of simple carbohydrates will be reported in another paper. The following points have been particularly emphasized:

1. The formation and mineralization of phosphorus in organic combination.
2. The relation of organic phosphorus to organic nitrogen during the process of decomposition.

EXPERIMENTAL

The materials selected for the study were alfalfa (*Medicago sativa*) and winter wheat (*Triticum vulgare*). Samples were taken at random from the border rows of the fertilizer experimental fields of the station. The plants were removed at the following stages of growth:

Young plant: alfalfa, 10–12 inches high; wheat, 12–14 inches high.
Medium plant: alfalfa, just before budding; wheat, just before heading.
Mature plant: wheat, after threshing.

The fresh materials, after being cleaned of weeds, were chopped into pieces one-half inch long and dried in the air. Known quantities of the air-dry materials were placed in glazed porcelain pots in duplicates or triplicates. Distilled water was added to give a moisture content of 70–80 per cent. For the mature wheat, mineral nutrients were added in addition to various amounts of dipotassium phosphate. A small amount of a 5 per cent suspension of fresh field soil was used to inoculate the materials. All pots were covered with glass plates, and the moisture content was maintained during the incubation. Samples were taken for analysis at different intervals.

For various determinations, appropriate duplicate aliquot portions of well-mixed decomposed material were introduced into weighed containers and reweighed, the difference being the amount of the sample taken for analysis. Total nitrogen, ammonia, nitrate, and total phosphorus were determined on the moist samples. The Kjeldahl method modified to include nitrate was used for total nitrogen, with mercury as a catalyst. Normal potassium chloride solution was used to extract the ammonia which was distilled with magnesium oxide. The nitrate was determined by reduction with Devarda's alloy on the potassium chloride extract after ammonia had been removed. The organic nitrogen was assumed to be the difference between the total nitrogen and the sum of the ammoniacal and nitrate nitrogen. The material for total phosphorus was ashed in the presence of magnesium nitrate and brought into solution according to the methods of A. O. A. C. (3). The volumetric method of A. O. A. C. was selected for the determination of the total and inorganic phosphorus. To extract inorganic phosphorus, Collison's method of HCl-alcohol extraction (5) was followed, 1 per cent HCl-alcohol being used instead of 0.2 per cent for the reasons suggested by Heck and Whiting (12). The moist samples for inorganic phosphorus determination were dried in flasks before extraction. The difference between the total phosphorus and the inorganic phosphorus was taken as the organic phosphorus. The ashing was made at 700°C. in an electic furnace. The results are expressed as averages of duplicates of material dried at 100°C.

Transformation of phosphorus during the decomposition of young and medium plant materials

In order to obtain the amount of material left or undecomposed, each pot was weighed before sampling, and the moisture content was determined. After the samples were taken, the pots were weighed again to give the amount of the material remaining for further decomposition. To calculate the amount of decomposition on the basis of the total material originally introduced into the pot, allowance was made for the material removed previously from each pot.

To correlate the transformation of phosphorus with the decomposition of plant materials a proximate chemical analysis of the materials was made according to the method of Waksman and Stevens (25), as shown in table 1.

No attempt was made to study the microflora developing during the decomposition. Occasional observations showed that certain species of *Rhizopus*, *Penicillium*, *Aspergillus*, and *Trichoderma* were the early invaders. The medium wheat was particularly rich in *Chaetomium*. As the decomposition proceeded further, the decomposed materials gradually became covered with white powdery patches. Microscopic examination revealed the white powder to consist of actinomycetes having long coiled aerial mycelium. These actinomycetes failed to grow on sodium albuminate agar (10, medium 5), but colonies developed with highly branched, straight, aerial mycelium.

Strong evolution of ammonia was detected in the young plant materials at the first period of sampling and in the medium alfalfa at the second sampling. The evolution persisted throughout the entire period of decomposition of these materials. Ammonia was never noted in the decomposition of medium wheat plant. From table 1 it will be noted that the medium wheat had a high content of energy-yielding material and a low nitrogen content. Such an unbalanced ratio leads to the assimilation of all of the available nitrogen.

Young wheat decomposed much more rapidly than any other materials. This is shown not only by the amount of the material decomposed but also by a complete loss of its structural identity. It is interesting to note that the medium plants decomposed more than the young plant materials during the first 14 days, but at 42 days it was the young plants which had undergone more decomposition.

Analyses show that 50–60 per cent of the total phosphorus of the plant materials studied consisted of organic forms of phosphorus. Young alfalfa

TABLE 1

Proximate chemical composition of young and medium alfalfa and wheat plants

(Per cent of dry material)

CONSTITUENT	ALFALFA		WHEAT	
	Young	Medium	Young	Medium
Cold-water-soluble organic matter	27.49	24.95	25.34	20.67
Hemicelluloses	10.50	10.86	17.56	18.61
Cellulose	14.04	18.77	17.27	22.45
Lignin	8.62	10.21	6.92	9.62
Total nitrogen	3.88	2.39	2.36	1.24
Ash	8.50	7.67	7.46	4.49

and wheat plants contained 0.35 per cent and 0.334 per cent total phosphorus, and the medium alfalfa and wheat had 0.227 per cent and 0.260 per cent phosphorus respectively. Because of the high content of nitrogenous materials in the alfalfa, the ratio of organic nitrogen to organic phosphorus is much higher in the legume than in the nonlegume. This ratio becomes lower as the plants get older.

The data presented in tables 2 and 3 reveal an extensive synthesis of organic compounds of phosphorus in the early stage of decomposition of all the plant materials. Except in the young alfalfa plant, the increase of the organic phosphorus content in per cent of total phosphorus was as much as 9 per cent over the content of the original material during the first 14 days of decomposition. There was greater conversion of inorganic phosphorus into organic compounds in the medium plant materials than in the young materials. As decomposition progressed further, more of the organic forms of phosphorus were broken down, resulting in lower contents of organic phosphorus in the residues.

Correspondingly, the percentage of the total phosphorus in the form of inorganic phosphorus increased.

TABLE 2

Transformation of phosphorus during the decomposition of young and medium alfalfa

INCUBATION	TOTAL DECOMPOSITION	TOTAL P PER CENT OF RESIDUE	INORGANIC P		ORGANIC P		ORGANIC N	ORGANIC N / ORGANIC P
			Per cent of residue	Per cent of total P	Per cent of residue	Per cent of total P	Per cent of residue	
days	*per cent*							
				Young plant				
0	0.0	0.357	0.169	47.34	0.188	52.66	3.754	19.97
14	23.17	0.473	0.215	45.45	0.248	54.55	3.880	15.04
28	42.07	0.565	0.243	43.01	0.322	56.99	3.580	11.12
42	54.16	0.729	0.355	48.70	0.374	51.30	4.018	10.74
				Medium plant				
0	0.0	0.227	0.093	40.84	0.134	59.16	2.274	16.97
14	33.01	0.355	0.113	31.83	0.242	68.17	2.958	12.22
28	42.04	0.392	0.139	38.01	0.243	61.99	3.290	13.57
42	45.54	0.417	0.195	46.76	0.222	53.24	3.402	15.32

TABLE 3

Transformation of phosphorus during the decomposition of young and medium wheat plants

INCUBATION	TOTAL DECOMPOSITION	TOTAL P PER CENT OF RESIDUE	INORGANIC P		ORGANIC P		ORGANIC N	ORGANIC N / ORGANIC P
			Per cent of residue	Per cent of total P	Per cent of residue	Per cent of total P	Per cent of residue	
days	*per cent*							
				Young plant				
0	0.0	0.334	0.144	43.11	0.190	56.89	2.321	12.22
14	31.30	0.478	0.160	33.47	0.318	66.53	2.970	9.34
28	51.74	0.662	0.251	37.92	0.411	62.08	3.842	9.35
42	58.03	0.760	0.364	47.89	0.396	52.11	4.590	11.59
				Medium plant				
0	0.0	0.260	0.127	48.85	0.133	51.15	1.164	8.75
14	37.46	0.443	0.158	35.67	0.285	64.33	1.801	6.32
28	51.68	0.538	0.200	37.18	0.338	62.82	2.552	7.55
42	55.85	0.582	0.230	39.52	0.352	60.48	2.964	8.42

In the course of the decomposition, the ratio of organic nitrogen to organic phosphorus was at first narrowed and then widened again.

It should be mentioned that an increase in the organic or inorganic phosphorus content in per cent of the residue does not necessarily mean an absolute

increase. This may be merely due to the disappearance of respirable materials and to the loss of volatile substances. If either the organic or the inorganic phosphorus is calculated as the percentage of total phosphorus or on the basis of the original material, there may actually be a decrease, as is clearly brought out in tables 2 and 3.

The formation and mineralization of organic phosphorus complexes seem to be affected by the amount of available energy-supplying materials, which are themselves influenced more by the age than by the nature of the plant. In the presence of an abundant supply of these energy-supplying materials, such as hemicelluloses and cellulose, and in the presence of nutrients, there is a vigorous microbial development, resulting in the synthesis of considerable amounts of organic phosphorus compounds at the expense of the available inorganic phosphates originally present in the material. The young wheat and the medium wheat and alfalfa have high contents of hemicelluloses and cellulose; after decomposition had proceeded for some time there was more organic phosphorus in these materials than in the young alfalfa. Furthermore, by reason of the fact that medium wheat was richer in hemicelluloses and cellulose, it maintained its high content of organic phosphorus longer than the young wheat plant during decomposition. When the materials which provide available energy become exhausted or the supply of available energy materials is limited, the microorganisms attack the complex organic compounds which contain phosphorus, including those originally present in the plant material and those present in the dead microbial cells. Part of the liberated phosphorus is reassimilated, while part of it is transformed into inorganic phosphates, resulting in an increase in the inorganic phosphorus content of the decomposed material. This increase will continue as long as additional energy-yielding material is withheld.

It is also interesting to note that, even during the early stages of decomposition of alfalfa, there is actually a temporary reduction in the amount of available phosphorus, while at the same time considerable amounts of available nitrogen are liberated. This suggests that the inorganic phosphorus in the plant material is in a readily convertible state and also that the organic compounds of phosphorus in the plant material are not readily transformed or that the microorganisms preferentially utilize the inorganic phosphorus. It is apparent that the transformation of phosphorus is affected by the supply of available energy material.

With rapid destruction of the nitrogenous substances and synthesis of organic phosphorus from the inorganic phosphorus, the ratio of organic nitrogen to organic phosphorus becomes narrower. With subsequent mineralization of the organic phosphorus compounds the ratio again becomes wide.

It is to be noted that accompanying the decomposition of such plant materials as young alfalfa and wheat the amount of organic phosphorus increases and the amount of inorganic phosphorus correspondingly decreases. This transformation has been consistently overlooked in the past. Although the

possibility existed that mineralization of phosphorus accompanies the liberation of ammonia during the decomposition of young plant material, this was not found to be the case. The increase in the amount of organic phosphorus at the expense of the inorganic phosphate during decomposition can be ascribed to the synthesis of cell material by the microorganisms effecting the decomposition.

Transformation of phosphorus during the decomposition of a mature wheat straw compost with different phosphorus contents

A series of 12 composts consisting of mature wheat straw were prepared in pots, each of which received 5 per cent calcium carbonate and 2.4 per cent ammonia sulfate and was inoculated with a dilute suspension of fresh field soil. The pots were then divided into four groups of three each and treated as follows:

I. No K_2HPO_4 added
II. 0.613 per cent K_2HPO_4 added
III. 1.225 per cent K_2HPO_4 added
IV. 2.450 per cent K_2HPO_4 added

The sulfate and phosphate were added in solution. The moisture content of the compost was maintained at 70–80 per cent with distilled water. The pots were covered and incubated at room temperature, or at 20°–23°C. Additional portions of 5 per cent calcium carbonate and 2.4 per cent ammonium sulfate were added to each pot after the first sampling. This was done in order to avoid excessive loss of ammonia which would have occurred if the total amount of the two compounds had been introduced at the beginning. Samples were taken after 14, 39, 67, and 104 days of composting. Representive samples from each treatment were prepared for chemical analyses by combining and mixing 50-gm. portions of each of the moist composts of three replicates.

In order to study the effect of phosphorus application upon the course of microbial decomposition of the straw, the evolution of CO_2 was followed according to the method of Waksman and Starkey (24). Three-gram portions of air-dry ground straw were placed in 300-cc. Florence flasks containing 100 gm. of sand (washed with conc. HCl and chromic acid cleaning solution), and to each flask was added 1 cc. of a 5 per cent suspension of field soil and 141.5 mgm. of ammonium sulfate dissolved in 5 cc. of water. Duplicate flasks received 5 cc. of one of the following phosphorus treatments:

I. No K_2HPO_4
II. 10 mgm. K_2HPO_4 (0.333 per cent by weight of straw)
III. 25 mgm. K_2HPO_4 (0.833 per cent by weight of straw)
IV. 101 mgm. K_2HPO_4 (3.367 per cent by weight of straw)

Two flasks containing the nutrients but no straw were inoculated and included as blanks. The moisture was brought up to 75 per cent. The flasks were connected to the aeration apparatus and incubated at 28°C. for 59 days; the

CO_2 was determined every day for the first 13 days, after aeration for 1 hour. The course of decomposition as affected by the phosphorus treatments is

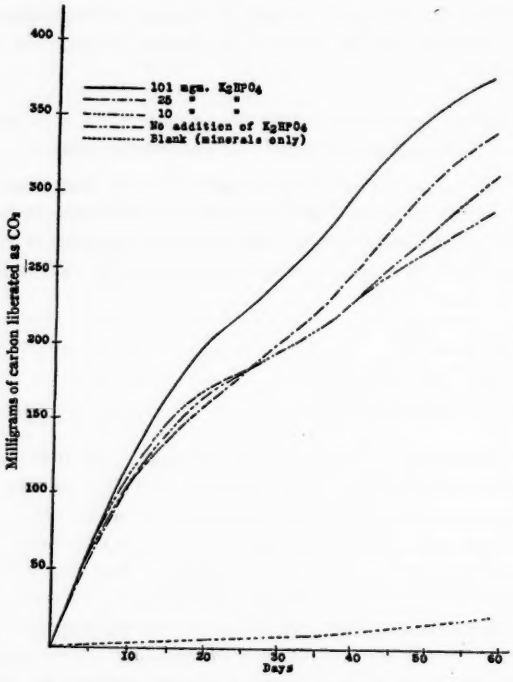

FIG. 1. EFFECT OF PHOSPHORUS UPON THE DECOMPOSITION OF MATURE WHEAT STRAW AS MEASURED BY CO_2 EVOLUTION

TABLE 4

*Effect of phosphorus upon the decomposition of mature wheat straw**

TREATMENT†	TOTAL P ADDED	INORGANIC P RECOVERED	ORGANIC P FOUND	TOTAL N ADDED	$NH_3 - N$ RECOVERED	ORGANIC N FOUND	$\frac{\text{ORGANIC N}}{\text{ORGANIC P}}$
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
No addition of P.....	2.4	0.0	2.4	42.4	20.5	21.9	9.13
10 mgm. K_2HPO_4.....	4.2	2.4	1.8	42.4	21.6	20.8	11.56
25 mgm. K_2HPO_4.....	6.9	3.2	3.7	42.4	18.3	24.1	6.51
101 mgm. K_2HPO_4.....	20.4	14.6	5.8	42.4	13.8	28.6	4.93

* 59 days of incubation.

† All flasks received 3 gm. ground air-dry straw + 141.6 mgm. $(NH_4)_2SO_4$.

shown graphically in figure 1, and the results of a chemical analysis of the residual materials are presented in table 4.

After a week of decomposition, the composts in all the flasks were covered with an abundance of heavy white fungus mycelium. *Chaetomium* sp. was found in all flasks during the early stage of decomposition.

After 39 days of decomposition, the pots receiving phosphate turned dark in color. The materials were friable and considerably decreased in volume. The state of decomposition of the materials is clearly indicated by the ash contents at 67 days as presented in table 5 and also by the course of the evolution of CO_2 as graphically shown in figure 1.

TABLE 5

Transformation of phosphorus during the decomposition of a mature wheat straw compost at different phosphorus contents

TREATMENT*	DECOMPOSITION PERIOD	TOTAL P PER CENT OF RESIDUE	INORGANIC P		ORGANIC P		ORGANIC N	ORGANIC N / ORGANIC P	ASH PER CENT OF RESIDUE
			Per cent of residue	Per cent of total P	Per cent of residue	Per cent of total P	Per cent of residue		
	days								
Original material.....	0	0.082	0.040	48.78	0.042	51.22	0.432	10.29	3.79
No K_2HPO_4.....	14	0.121	0.054	44.63	0.067	55.37	1.056	15.76	10.79
	39	0.128	0.039	30.47	0.089	69.53	1.450	16.29	15.50
	67	0.151	0.037	24.51	0.114	75.49	1.790	15.70	18.23
	104	0.154	0.048	31.17	0.106	68.83	1.870	17.67	20.96
0.613 per cent K_2HPO_4	14	0.242	0.126	52.07	0.116	47.93	1.220	10.52	11.10
	39	0.265	0.129	48.68	0.136	51.32	1.520	11.18	16.68
	67	0.310	0.151	48.71	0.159	51.29	1.840	11.57	20.51
	104	0.325	0.181	55.69	0.144	44.31	1.900	13.19	21.80
1.225 per cent K_2HPO_4	14	0.394	0.272	69.04	0.122	30.96	1.097	8.99	11.92
	39	0.417	0.247	59.23	0.170	40.77	1.560	9.18	16.78
	67	0.466	0.311	66.74	0.155	33.26	1.800	11.61	21.01
	104	0.483	0.343	71.02	0.140	28.98	1.780	12.71	22.67
2.450 per cent K_2HPO_4	14	0.689	0.507	73.58	0.182	26.42	1.150	6.32	13.18
	39	0.714	0.476	66.67	0.238	33.33	1.680	7.06	19.45
	67	0.807	0.624	77.32	0.183	22.68	1.820	9.94	23.22
	104	0.864	0.690	79.86	0.174	20.14	1.830	10.52	24.26

* In addition to 10 per cent $CaCO_3$, 4.8 per cent $(NH_4)_2SO_4$, etc.

From table 5 it will be observed that the addition of inorganic phosphate greatly stimulated the decomposition of the mature wheat straw and also led to an accumulation of organic phosphorus. For example, at 39 days the organic phosphorus content of the original straw was raised from 0.042 per cent to 0.089 per cent in the residue receiving no phosphate, to 0.136 per cent in the residue receiving 0.613 per cent phosphate, to 0.170 per cent in the residue receiving 1.225 per cent phosphate, and to 0.238 per cent in the residue receiving 2.45 per cent phosphate.

The synthesis of organic phosphorus is further indicated by the narrowing of the ratios of organic nitrogen to organic phosphorus, namely, from 10.29 in the original material to 6.32 in the material receiving 2.45 per cent phosphate. This ratio became wider with each material as decomposition progressed.

The content of organic phosphorus calculated in per cent of total phosphorus was highest in the compost receiving no addition of phosphate, even after 104 days, indicating in this case that the active microorganisms converted the inorganic phosphorus originally present in the plant material into organic phosphorus and that the organic compounds of phosphorus were inadequate for the requirements of the microorganisms. This emphasizes further that when energy-supplying material is present in abundance, phosphate is assimilated and transformed into organic compounds which accumulate as long as the energy material and the inorganic phosphate do not become exhausted.

It is noted that a small addition of phosphate has little effect on the rate of decomposition, whereas a large dose greatly affects the rate of decomposition; the effect becomes more apparent after long periods of incubation. This effect is further illustrated by the results of chemical analyses presented in table 4, which show that considerable amounts of both organic phosphorus and organic nitrogen were synthesized.

After 104 days the reaction of the residues remaining in all of the pots was close to pH 6.4.

Decomposition of various organic constituents of mature wheat straw at different phosphorus contents

One-hundred-gram portions, calculated on the oven-dry basis, of finely cut mature wheat straw were introduced into 2-liter weighed flasks. To each flask were added 5 cc. of a dilute suspension of fresh soil and 4.72 per cent ammonium sulfate. Duplicate flasks were given one of the following treatments:

I. No K_2HPO_4
II. 0.337 per cent K_2HPO_4
III. 1.236 per cent K_2HPO_4
IV. 1.236 per cent K_2HPO_4 + 9 per cent $CaCO_3$
V. 3.372 per cent K_2HPO_4
VI. 5.131 per cent K_2HPO_4

In addition to these treatments two flasks containing straw alone were inoculated and included in the study. Water was added to the composts to give a total amount equivalent to 300 per cent of the oven-dry weight of the straw. All materials were incubated at 28°C. Samples were taken for study after 60 and 116 days.

The flasks were weighed before and after sampling to ascertain the material left after decomposition and also the material remaining for further decomposition. After each material was thoroughly mixed, aliquot portions were removed for various determinations. Moist samples equivalent to 5 gm. of dry

material were taken for the proximate analysis. The results, calculated on the basis of percentage of the original material, are recorded in tables 6 and 7.

Certain species of *Rhizopus*, *Aspergillus*, *Trichoderma*, *Penicillium*, and *Chaetomium* were found in abundance in all of the composts, the last two species being most numerous. Development of fungi was not nearly so extensive in the flasks containing the straw alone as in the treated flasks.

TABLE 6

Proximate chemical composition of a mature wheat straw compost at different phosphorus contents

(On the basis of original straw)

TREATMENT*	DECOMPOSITION PERIOD	TOTAL DECOMPOSITION	COLD-WATER-SOLUBLE ORGANIC MATTER	HEMICELLULOSES	CELLULOSE	LIGNIN
	days	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Original straw	0	0	5.53	26.80	35.49	16.84
Straw only	60	23.74	3.48	18.13	23.37	15.57
	116	26.52	3.98	16.19	19.67	13.71
Minerals, no P	60	25.98	4.87	16.48	18.56	17.11
	116	30.38	4.41	13.51	15.51	17.81
0.337 per cent K_2HPO_4	60	25.02	4.95	17.49	18.22	17.72
	116	30.89	4.66	13.52	13.62	17.35
1.236 per cent K_2HPO_4	60	25.55	4.55	17.70	16.75	16.11
	116	37.88	4.25	12.10	12.30	17.31
1.236 per cent K_2HPO_4 + 9 per cent $CaCO_3$	60	35.89	2.17	9.41	8.76	16.53
	116	39.50	1.87	7.50	6.16	14.79
3.372 per cent K_2HPO_4	60	32.45	3.79	13.26	14.06	16.23
	116	38.12	3.59	9.90	9.63	17.14
5.131 per cent K_2HPO_4	60	34.40	3.58	11.77	12.57	15.97
	116	43.45	3.38	8.41	7.27	15.10

* In addition to 1 per cent N as $(NH_4)_2SO_4$.

The reaction (pH) of the residues after 116 days was as follows (determined by use of glass electrode): I. 4.05, II. 4.15, III. 4.40, IV. 6.10, V. 4.35, VI. 5.05.

Some interesting facts are brought out by the results in table 6. In general, the addition of phosphate markedly increased the decomposition of various organic constituents of the straw. Whereas the amounts of cold-water-soluble organic matter and lignin were only slightly changed, the hemicelluloses and cellulose suffered pronounced losses, and decomposition was greater where large amounts of phosphate were added. The greatest apparent effect of the phosphate was on the cellulose. The cellulose content was reduced from 35

per cent in the original straw to 7.27 per cent in the compost receiving 5.131 per cent phosphate, after 116 days of decomposition. Hemicelluloses were reduced from 26.80 per cent to 8.41 per cent. Materials receiving small amounts of phosphate did not differ much among themselves or from the compost of straw receiving only nitrogen; the decomposition of the untreated straw was considerably slower than that of any of the treated materials. The

TABLE 7

Transformation of phosphorus during the decomposition of a mature wheat straw compost at different phosphorus contents

TREATMENT*	DECOMPOSITION PERIOD	TOTAL RESIDUE	TOTAL P PER CENT OF RESIDUE	INORGANIC P		ORGANIC P			ORGANIC N PER CENT OF RESIDUE	ORGANIC N / ORGANIC P	ASH PER CENT OF RESIDUE
				Per cent of residue	Per cent of total P	Per cent of residue	Per cent of total P	Per cent of original straw			
	days	*per cent*									
Original straw	0	100.00	0.082	0.040	48.78	0.042	51.22	0.042	0.432	10.29	3.79
Straw only	60	76.26	0.098	0.047	47.96	0.051	52.04	0.038	0.580	11.37	5.07
	116	73.48	0.119	0.047	39.49	0.072	60.51	0.052	0.770	9.31	6.57
Nutrients, no P	60	74.02	0.110	0.040	36.36	0.070	63.64	0.052	0.880	12.57	6.40
	116	69.62	0.127	0.044	34.64	0.083	65.36	0.057	1.170	14.01	7.63
0.337 per cent K_2HPO_4	60	74.98	0.165	0.092	55.76	0.073	44.24	0.055	0.900	12.33	6.88
	116	69.11	0.212	0.115	54.24	0.097	45.76	0.067	1.260	12.99	8.09
1.236 per cent K_2HPO_4	60	74.45	0.382	0.243	63.52	0.139	36.48	0.104	1.180	8.49	8.18
	116	62.12	0.483	0.359	74.33	0.124	25.67	0.077	1.410	11.37	9.91
1.236 per cent K_2HPO_4 + 9 per cent $CaCO_3$	60	64.11	0.443	0.206	46.50	0.237	53.50	0.152	0.152	8.61	20.68
	116	60.50	0.471	0.267	56.69	0.204	43.31	0.124	2.120	10.39	22.63
3.372 per cent K_2HPO_4	60	67.55	0.963	0.719	74.66	0.244	25.34	0.165	1.590	6.51	11.32
	116	61.88	1.107	0.879	79.40	0.228	20.60	0.141	1.690	7.41	12.22
5.131 per cent K_2HPO_4	60	65.60	1.485	1.038	69.90	0.447	30.10	0.293	1.740	3.89	15.29
	116	56.55	1.639	1.226	74.80	0.413	25.20	0.234	1.860	4.51	16.35

* In addition to 1 per cent N as $(NH_4)_2SO_4$.

addition of calcium carbonate brought about the greatest decomposition of the water-soluble organic matter, hemicelluloses, and cellulose, not excepting the material receiving the greatest amount of phosphate.

The fact that large amounts of inorganic phosphate may be converted into organic phosphorus, during the decomposition of organic matter rich in energy-yielding material, is further emphasized by the results of table 7. After 60 days of decomposition, in the presence of 5.131 per cent dipotassium phosphate, the content of organic phosphorus was seven times the original amount. Anal-

yses of straw receiving no phosphate or only a small amount showed evidence of synthesis of organic phosphorus but no mineralization during the 116 days. There was appreciable mineralization of organic phosphorus at 116 days only in the composts containing large amounts of phosphate. This may be ascribed to the rapid decomposition of these materials. Calcium carbonate exerted a favorable influence on the decomposition of the straw. More inorganic phosphate was converted into organic phosphorus in the limed straw than in the unlimed straw receiving the same amount of phosphorus. The increase of organic phosphorus can be noted indirectly from the ratio of organic nitrogen to organic phosphorus.

The results obtained from the decomposition of medium wheat plants as well as from the last experiments strongly emphasize the fact that, in the presence of large quantities of energy-yielding material, a considerable amount of inorganic phosphorus is transformed into organic phosphorus. Furthermore, evidence of mineralization appears first in those materials from which the readily decomposable substances are most quickly exhausted.

SUMMARY

A study has been made of the transformation of phosphorus during the microbiological decomposition of alfalfa and wheat at three stages of growth; namely, young, medium, and mature.

In the early stages of decomposition of the plant materials a considerable amount of organic phosphorus was synthesized by microorganisms at the expense of the inorganic phosphorus. This is true irrespective of the nature and the stage of growth of the plants. As decomposition progressed further, the organic complexes of phosphorus were gradually mineralized, the rate of which depended upon the stage of growth rather than upon the nature of the plant. For example, at 42 days the contents of organic phosphorus of the young alfalfa and wheat and the medium alfalfa dropped slightly below their original values, whereas that of the medium and mature wheat remained above the original content. The increases and the decreases of the organic phosphorus were accompanied by corresponding decreases and increases of inorganic phosphorus.

The addition of dipotassium phosphate to mature straw compost markedly increased the decomposition of the straw, as shown by the amount of material decomposed and by the rate of CO_2 evolution.

More inorganic phosphorus was transformed to organic forms during the decomposition of old materials than during the decomposition of young materials. As much as seven times the original content of organic phosphorus was found in straw compost receiving 5.131 per cent K_2HPO_4 for 60 days of incubation. In straw receiving no phosphate or only a small amount of phosphate there is no evidence of mineralization of the organic phosphorus during 116 days of decomposition. Appreciable mineralization of organic phosphorus was apparent only in materials treated with large amounts of phosphate.

The fractions of the straw most affected by the phosphate applications appear to be the cellulose and hemicelluloses. The decomposition of both fractions was considerable where phosphate was added, whereas the amounts of cold-water-soluble organic matter and lignin were virtually unchanged during the period of decomposition.

The synthesis of organic phosphorus can be further shown by a narrowing of the ratio of organic nitrogen to organic phosphorus. The ratio decreases and increases with the formation and mineralization of organic phosphorus respectively.

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STUDIES IN THE NUTRITION OF VEGETABLES: THE EFFECTS OF VARYING THE POTASH SUPPLY ON SAND CULTURES OF LETTUCE

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Two experiments were made to study the effects of varying the potash supply on sand cultures of lettuce. The plants were grown in fine, white, pure silica sand, described elsewhere (2), seven media being used in each experiment.

The concentrations of important elements present in the media are given in table 1. The salts used in making the media were as follows: sodium nitrate, *AnalaR*, as the source of nitrogen; anhydrous disodium phosphate, *AnalaR*, as the source of phosphorus; potassium sulfate, *AnalaR*, as the source of potassium (and sulfur); a solution of calcium chloride made by neutralization of calcium carbonate, *AnalaR*, with hydrochloric acid, as the source of calcium; magnesium sulfate heptahydrate, *AnalaR*, as the source of magnesium (and sulfur); and ferrous sulfate heptahydrate, *AnalaR*, as the source of iron (and sulfur). The minor elements were found to be unnecessary in these cultures. It will be observed that, for experiment 1, the first six media (A-F) were identical except for a variation in the potassium sulfate content, and that the seventh medium (G) contained potassium and ferrous sulfates only. For experiment 2, the "potash-alone" treatment was omitted and the "no-potash" treatment was expanded to an otherwise complete treatment (GI).

Glazed culture jars, each holding about 46 pounds of sand, were used in the first experiment. In the second experiment, as glazed culture jars were not available, ordinary plant pots holding about 13 pounds of sand were used; the pots were coated outside with three coats of copal varnish and then immersed in boiling "refined" paraffin (m.p. = 110°F.) for 30 minutes.

The lettuce, variety May King, was sown in the sand in the culture jars and pots so that the seed germinated in contact with the medium and there was no transplanting to check growth and develop the coloration of this tinted lettuce (3).

In experiment 1, started on May 25, 1936, there were 12 replications of each of the first five treatments A–E, and the 60 cultures were arranged in an unheated greenhouse as a "layout" of 12 randomized blocks. There were only three cultures each of treatments F (no potash) and G (potash and iron alone),

[1] I thank my assistants T. W. McKean and J. F. Leonard for their great help in the routine of these experiments.

and consequently these six cultures were left outside the randomization. During the experiment, 15 liters of medium were supplied to each culture, usually in 500-cc. lots on alternate days. As medium E yielded seedlings of about the same size as those from treatments A, B, C, and D, which all contained more potash, the amount of potash present in medium E was reduced on June 17 to 5.61 p.p.m., on June 26 to 2.81 p.p.m., and on July 1 to 1.12 p.p.m.; the resulting concentrations of sulfur then present in E were 29.23, 28.08, and 27.39 p.p.m.

The second experiment was begun on June 30, 1937. In the randomized arrangement the seven treatments were replicated 16 times, making 112 cultures. During this experiment 8½ liters of medium were applied to each cul-

TABLE 1

Concentration of certain elements in the media

MEDIA	N	P	K	Ca	Mg	Fe	S	Na
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Experiment 1								
A	16.48	43.70	89.76	36.12	20.20	0.50	63.73	91.88
B	16.48	43.70	67.32	36.12	20.20	0.50	54.53	91.88
C	16.48	43.70	44.88	36.12	20.20	0.50	45.33	91.88
D	16.48	43.70	22.44	36.12	20.20	0.50	36.13	91.88
E	16.48	43.70	11.22	36.12	20.20	0.50	31.53	91.88
F	16.48	43.70	0	36.12	20.20	0.50	26.93	91.88
G	0	0	44.88	0	0	0.50	18.69	0
Experiment 2								
AI	16.48	5.46	44.88	18.06	5.05	0.50	25.35	35.18
BI	16.48	5.46	22.44	18.06	5.05	0.50	16.15	35.18
CI	16.48	5.46	16.83	18.06	5.05	0.50	13.85	35.18
DI	16.48	5.46	11.22	18.06	5.05	0.50	11.55	35.18
EI	16.48	5.46	5.61	18.06	5.05	0.50	9.25	35.18
FI	16.48	5.46	0.56	18.06	5.05	0.50	7.18	35.18
GI	16.48	5.46	0	18.06	5.05	0.50	6.95	35.18

ture, usually in 250-cc. lots three times a week. As the low phosphate content of the media tended to produce purple flushes as the lettuce matured (2, 3), the sodium phosphate content was quadrupled on August 16, so that the media all contained 21.85 p.p.m. of phosphorus. The cultures rapidly became green again (3).

OBSERVATIONS ON THE CULTURES

May 25, 1936: seeds sown for the first experiment; June 3, 1936: germination complete; June 3, 1936: the seedlings were singled; July 10, 1936: harvest.

June 30, 1937: seeds sown for the second experiment; July 6, 1937: germination complete; July 8, 1937: the seedlings were singled; August 31, 1937: harvest.

Summaries of the notes made during the experiments follow:

The seedlings at germination in the first experiment were green; in two days with G (potash alone) the seedlings were darker and tinged with red at the edge. As the experiment progressed, these plants were the darkest of all the cultures, but later became dull bronze. The plants were stunted, with flat, smooth leaves, and had a typical flat, rosette appearance.

During growth a slight deepening in shade developed in passing from A–F, although the green of the F cultures (no potash) was never so dark as that of G. Ultimately the cultures with F turned chlorotic green.

After about 3 weeks the cultures with F (no potash) had a very characteristic appearance. The leaves were smaller than those of normal plants and had wider veins. Likewise, they were not so flat; this was not due to a wavy leaf as a whole, but to the fact that the portions between the veins all tended to be convex on the upper surface, so that a "crinkly" and typically "starved" appearance resulted. These convex portions were darker green than was normal; but later, as previously stated, the plants became rather chlorotic in appearance. Finally the leaf margins also tended to curl upward. As a result of scorch the cultures with F presented a typically tall, narrow appearance.

In the second experiment not much difference in tint was noticed among the cultures. The treatments with least potash gave plants of an abnormally erect habit of growth, as a result of scorch.

Scorch appeared in the first experiment on cultures with E and F after about 4 weeks. Brown dead patches (showing up the network of the leaf structure) and marginal scorch appeared on the outer leaves. These symptoms were followed by limpness and finally by absolute withering of the whole leaf. The scorch due to this deficiency or absence of potash always worked inward from the oldest leaves toward the heart. All the E cultures were severely attacked, but the disappearance of the outer leaves of some of the cultures left lettuce which could be placed in Grade I at harvest. The cultures with F (no potash) were so consistently and badly affected, and the outer leaves withered so rapidly, that all growth seemed to be upward, and a characteristically high, narrow, erect plant finally resulted, as has already been mentioned.

In the second experiment, somewhat similar effects were obtained, but earlier, especially with treatments EI, FI, and GI. Similar marginal scorch extended inward, and patches of scorched tissue broke away and left holes in the leaves. As the older leaves withered and died off, the plants were, at times, not so poor in appearance. The treatment without potash (GI) did not lead to such immature, small, erect plants as those in the first experiment, possibly because, as perceived from the size and weight, the plants receiving treatment GI must have obtained some potash from somewhere. As "spot" tests failed to show the presence of potash in the sand, this potash might have come from the plant pots which, at the end of the experiment, were found to be wetted by water despite the boiling in wax that they had undergone; or

alternatively the sodium present might have replaced potassium to some extent.

Linear measurements of each culture, that is, the greatest spread and the breadth of the culture at right angles to this, were made weekly. At no time was there much difference with treatments A–E in the first experiment. Treatments F and G, however, gave small cultures. At harvest the means of the measurements, in centimeters, were as follows: A, 22.3 x 20.9; B, 22.2 x 21.4; C, 21.4 x 19.8; D, 21.9 x 20.7; E, 22.6 x 21.3; F, 8.3 x 5.7; and G, 3.0 x 2.0. For the first 3 weeks in the second experiment the treatments with least potash gave the smallest cultures. As the experiment progressed, the sizes became more uniform, until at harvest they were, in centimeters, as follows: AI, 26.1 x 24.1; BI, 25.7 x 23.3; CI, 25.7 x 23.7; DI, 25.0 x 22.8; EI, 25.4 x 23.3; FI, 25.0 x 22.5; and GI, 25.1 x 22.7. The results with GI demonstrated that these cultures were obtaining potash, possibly from the pots, as previously explained. In general, the results confirmed those for the first experiment in the important point that potash over a wide range had apparently little effect on size.

The numbers of lettuce plants which were fully hearted and those which were hearting up (the latter values are in brackets) with the various treatments in the first experiment at harvest follow: A, 9 [3]; B, 6 [6]; C, 7 [5]; D, 7 [5]; E, 8 [4]; F, 0 [0]; G, 0 [0]. Thus, increasing the dose of potash beyond that in E (which showed potash-deficiency symptoms as regards scorch, if not as regards size and weight) did not cause earlier maturity. Corresponding figures for the second experiment at harvest were as follows: AI, 4 [8]; BI, 5 [10]; CI, 4 [10]; DI, 4 [12]; EI, 3 [13]; FI, 0 [16]; and GI, 1 [13]; in addition, 4 of AI and 1 each of BI and CI had flower stems forming, which stopped hearting. The conclusion as regards maturity, drawn from the first experiment, was therefore confirmed.

With A, B, C, D, and E, in the first experiment, the roots were normal, well-developed, and bushy taproots; F gave a small, undeveloped root lacking in fiber; and G gave the smallest roots, with long, coarse, hairlike side-shoots. Similar results were obtained in the second experiment, except that FI and GI were about half the size of the roots with treatments AI–EI.

The numbers of lettuce heads judged to be of first grade at harvest in the first experiment were: A, 9; B, 6; C, 7; D, 7; E, 3; F, 0; G, 0. With A, B, C, and D, the remaining heads at harvest were spoilt by the appearance of flower stems or by immaturity; with E, scorch also was responsible to a great extent. Corresponding results were obtained for the second experiment.

YIELD WITH DIFFERENT TREATMENTS

The data for each culture were too bulky to be reproduced but are available for examination in this laboratory. The analyses of variance (1, p. 15 *et seq.*) were worked out for the fresh and dry weights of the tops (cut off at the junction of top with stalk), roots, and total plants, the top/root ratios for fresh and dry weights, and the percentages of moisture in the tops, roots, and total

plants. The corresponding summaries of results for the two experiments are given in tables 2 and 3, together with the standard errors of the treatment means (1, p. 15 *et seq.*). Arithmetical, not weighted, means have been used when dealing with ratios and percentages (3).

In the first experiment the results for the fresh weights of the tops and total plants were not significant. The weights for the roots, however, were highly significant, although even here the results with media A, B, and C, were significantly equal, and the potassium had to be reduced to 22.44 p.p.m. before an increased root system resulted. It was evident that the fresh weights of the plants were unaffected over a wide range of potassium concentrations, the main response being with the roots, where more potassium than the optimum, 22.44 p.p.m., decreased growth. That some potassium was necessary for good

TABLE 2

Summaries of results of experiment 1

DESCRIPTION OF DATA	TREATMENT MEAN FOR					MEAN OF ALL RESULTS	S.E.	F	G
	A	B	C	D	E				
Tops, FW., *NS*.	32.37	34.41	25.93	32.31	29.84	30.97	2.073	4.964	0.112
Roots, FW., *SS*.	8.466	10.03	10.26	12.45	11.09	10.46	0.7018	0.257	0.075
Total plants, FW., *NS*.	40.84	44.44	36.19	44.76	40.92	41.43	2.466	5.221	0.186
Tops, DW., *NS*.	2.568	2.772	2.325	2.900	2.713	2.656	0.1326	0.375	0.026
Roots, DW., *NS*.	1.289	1.276	1.323	1.596	1.409	1.379	0.1042	0.024	0.021
Total plants, DW., *NS*.	3.857	4.048	3.648	4.497	4.123	4.034	0.2244	0.399	0.047
Top/root, FW., *SSS*.	3.864	3.415	2.654	2.635	2.698	3.053	0.1998	19.08	1.484
Top/root, DW., *NS*.	2.014	2.180	1.784	1.941	1.928	1.970	0.1179	14.80	1.265
Tops; per cent moisture, *SSS*.	92.01	91.85	91.11	90.94	90.86	91.36	0.2102	92.27	76.19
Roots; per cent moisture, *SS*.	84.81	87.27	86.85	87.29	87.24	86.69	0.4932	89.76	72.14
Total plants; per cent moisture, *S*.	90.52	90.82	90.00	89.88	89.88	90.22	0.2471	92.13	74.55

growth of both tops and roots was clearly shown by the results with F (no potash); here absence of potash resulted in small tops, roots, and total plants.

In these experiments the potash applied was available to the cultures. As it is generally understood that comparatively large quantities of potash should be applied to produce good growth of this crop (apart altogether from their use from other considerations, such as resistance to frost and scorch), it can only be assumed that these amounts are necessary because potash is not usually readily available after admixture with the soil.

The corresponding results for the second experiment were highly significant, although here also the results with media AI, BI, and CI, were significantly equal for tops, roots, and total plants, which demonstrated lack of response to potash over a wide range, 44.88–16.83 p.p.m. The decrease only commenced with DI (11.22 p.p.m.); and the roots even with EI (5.61 p.p.m.) were very

good and were statistically equal to those for CI and DI. Although FI and GI were statistically equal, and both were significantly less than for all the other treatments for tops, roots, and total plants, they were still substantially large, and demonstrated that such small amounts of potash as could be derived from the pots and/or sand, even after immersion of the pots in boiling paraffin,

TABLE 3
Summaries of results of experiment 2

DESCRIPTION OF DATA	TREATMENT MEAN FOR							MEAN OF ALL RESULTS	S.E.
	AI	BI	CI	DI	EI	FI	GI		
Tops, FW., *SSS*.....	45.34	44.98	42.96	39.06	38.58	32.23	33.49	39.52	0.9333
Roots, FW., *SSS*.....	16.82	16.81	14.75	11.87	12.48	6.449	7.514	12.39	0.8191
Total plants, FW., *SSS*.....	62.16	61.79	57.71	50.93	51.07	38.67	41.00	51.90	1.618
Tops, DW., *SSS*.....	2.313	2.294	2.285	2.036	2.028	1.479	1.628	2.009	0.0870
Roots, DW., *SSS*.....	1.336	1.151	1.151	0.904	0.976	0.488	0.472	0.9254	0.0921
Total plants, DW., *SSS*.....	3.649	3.445	3.436	2.941	3.004	1.968	2.100	2.934	0.1505
Top/root, FW., *SSS*.....	2.85	2.74	2.98	3.46	3.32	6.61	5.26	3.889	0.4142
Top/root, DW., *SSS*....	2.03	2.05	2.13	2.44	2.47	4.27	4.26	2.808	0.3162
Tops; per cent moisture, *S*.....	94.89	94.90	94.69	94.79	94.75	95.39	95.15	94.94	0.1659
Roots; per cent moisture, *NS*.....	92.20	93.13	92.29	92.47	92.48	92.66	93.79	92.72	0.3655
Total plants; per cent moisture, *SSS*.....	94.15	94.43	94.07	94.25	94.17	94.96	94.91	94.42	0.1749

Notes: In the first column, "Description of Data," FW and DW denote fresh and dry weights; *S*, *SS*, *SSS*, or *NS*, indicates that the treatment means in the unpublished tables of analyses of variance, according to Fisher's tables of *z*, were significant at the 5, 1, and 0.1 per cent levels, or were not significant, respectively.

The treatment means are recorded under the "Treatment Mean for" column subheadings. The mean of all the results for a subtable (which is also the mean of the treatment means) is given in the next column; the standard errors of the treatment means are recorded in the column headed "S.E." The difference between any two of the treatment means in a subtable was taken to be significant when it exceeded three times the S.E.

The "no-potash" (F) and "potash-alone" (G) results for the first experiment are averages of three cultures for each treatment, and are tabulated in the two columns after the column "S.E."; as only the results for treatments A-E were included in the randomized block in this experiment, the mean and S.E. apply only to these five treatments, and not to F and G.

were sufficient for moderately good growth, or that sodium could replace the missing potassium.

It was worthy of note that in both experiments a deficiency of potash demonstrated itself by serious scorch before the deficiency was so great as to lead to a serious diminution in growth.

Somewhat similar results were obtained for the dry weights as for the fresh weights, and there was again a marked lack of response to potash over wide

ranges of concentration. Thus for the tops, roots, and total plants, the results with media AI, BI, and CI were statistically equal in the second experiment, and in the first the corresponding results were not significant (NS). In the second experiment, results with FI and GI were statistically equal, and both were significantly smaller than those with all other treatments in the three comparisons, but even then they were substantially large. The results with F (no potash) for the first experiment, however, demonstrated that absence of potash resulted in stunted growth and correspondingly small dry weights for tops, roots, and total plants.

With the fresh weights, the differences in the top/root ratios for the first experiment were highly significant and demonstrated that, in general, the tops were favored more by increased potash than were the roots, the weights of which were actually increased by reduction in potash to the optimum amount of 22.44 p.p.m.; the corresponding ratios for the dry weights were not significant. In the second experiment the results were highly significant for both the fresh and dry weights and showed that although over a wide range, AI–EI, the ratios were statistically equal (thus going a long way toward bearing out the results of the first experiment), the very small quantities of potash provided by the treatments FI and GI favored the tops rather than the roots.

The results for the moisture contents in the first experiment were highly significant for the tops and roots and significant for the total plants. With the tops, decrease in potash supply below B resulted in a decrease in the moisture content; with the roots the provision of more potash seemed to give no response over a wide range (B–E), but ultimately (treatment A) led to a decrease. The results of the second experiment for tops and total plants (the results with the roots were not significant) also tended to prove the lack of response in this respect over wide ranges of concentration; here a very low concentration of potassium, FI = 0.56 p.p.m., was found to give the highest moisture content, and GI also gave, both for the tops and total plants, results statistically equal to those of FI.

SUMMARY

May King lettuce grown in sand showed very little response to potassium over a wide range of concentration. Small amounts gave moderately good growth, although a complete absence of potash resulted in a small plant. The ready availability of the potassium supplied in culture experiments is possibly responsible for the small amount required as compared with the comparatively large amounts recommended for growth of the plants in soil.

Lack of potash first gave a slightly darker leaf than normal, but later tended to cause chlorosis. Scorch appeared with small amounts of potash, even when these were not small enough to cause an appreciable reduction in size. Absence of potash resulted in such rapid, severe scorch and withering away of the older leaves that ultimately a characteristically small, narrow, relatively tall and erect plant resulted; the portions of the leaves between the abnormally

wide veins were convex on the upper surface and gave the leaves a characteristic wavy appearance; these areas were at first darker green than normal, but later became chlorotic.

Large applications of potash did not induce earlier hearting.

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THE INFLUENCE OF VARYING CATION PROPORTIONS UPON THE GROWTH OF YOUNG COTTON PLANTS

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Investigators seeking an insight into the relationships between soil chemistry and plant response have frequently discounted the value of plant nutrient solution studies. This attitude may be justified when one considers that the disparity between the concentration of chemicals in most nutrient solutions and that of a soil solution is of sufficient magnitude to preclude any comparison. For example, the R_2S_4 solution (4) at one atmosphere concentration which has been widely used in nutrient culture work, has the following concentration of nutrient elements in comparison to the available content of these elements in extracts of productive soils (6) giving high microchemical tests for these same elements:

	R_2S_4 SOLUTION (1.0 ATMOS.)	SOIL EXTRACT HAVING HIGH READINGS
	p.p.m.	*p.p.m.*
K	175	50
Ca	360	200
Mg	108	12
PO_4-P	140	5
NO_3-N	252	25
SO_4-S	144	25

When one bears in mind that these values for the soils represented *available* nutrients and not just those actually in the soil solution, the differential is even greater. And even though one element in such a nutrient solution was maintained at a level comparable to that existing in soils, it is difficult to conceive that the disparity between the two conditions in the concentrations of other elements present would be without effect upon plant response. It would seem, also, that when a technic of continuous solution renewal is used, the concentration of the nutrient ions should more nearly approach that of the soil solution if any parallelism in the nutritional effects is to be studied.

Furthermore, the degree of active acidity in a nutrient solution must be differentiated in interpreting its effect upon plant response from soil conditions giving a similar acidity reading. Other conditions being equal, plants may be grown in nutrient solutions varying as to pH over a considerable range (pH 4–7) without inducing any significant difference in growth response, and yet

show marked differences in growth in soils adjusted over this same range. Solution culture eliminates the vagaries of the exchange capacity of the colloidal complex of a soil. Thus, a soil with an acidity reading of pH 4.0 indicates that the major portion of such exchangeable bases as K^+, Na^+, Ca^{++}, and Mg^{++} have leached out, establishing a new equilibrium in the colloidal complex in which there is an increasing saturation with H^+, thereby inducing Fe^{+++} and Al^{+++} to enter into the exchange complex in increasing quantities. In fact, these phenomena form the basis for the thiocyanate test for soil acidity, i.e., increasing $[H^+]$ concentration is associated with increasing solubility of tribasic cations. Most nutrient solutions are so constituted that Fe and Al are either absent or present only in relatively minute quantities. Thus, similar hydrogen-ion activities in the two types of substrates may not have parallel effects.

Most nutrient solutions which have been used in physiological balance studies have been calculated on the molecular basis. For the most part, this has been satisfactory, but it has led to difficulties when one wished to introduce a substitution of salts in deficiency studies. Thus, a mol of KNO_3 introduces only one-half as much nitrogen as a mol of $Ca(NO_3)_2$ when the former substitutes the latter in calcium deficiency studies. Furthermore, in many of these nutrient solutions, changes in the cation proportions were accompanied by corresponding changes in anion proportions, making for difficulty in the interpretation of ionic effects. Beckenbach et al. (2) devised a system of nutrient solutions in which the anions and cations were varied independently and thus avoided any such contingency. Unfortunately, however, their nutrient solutions were calculated on the molecular basis rather than the equivalence basis. Since stoichiometrical relationships of ions cannot be ignored in any study involving mineral absorption by plants, it would seem reasonable that the ionic concentration of the external medium should be expressed on the equivalence basis.

In order to take into account the aforementioned considerations, a system of devising nutrient solutions was developed wherein the total ionic concentration was maintained at a comparatively low level, ionic proportions were calculated on the equivalence basis, and the ferric ion was introduced as one of the variable components to aid in simulating soil acidity conditions arising from desaturation of exchangeable bases. It is not suggested that any of the solutions used were "ideal." Although excellent growth of the cotton plant was produced by some of these solutions, it must be conceded that modifications of the solutions might have produced more plant growth. But the value of the study lies not in the searching of a "theoretical ideal," but in the observations of the physiological response of a species to a definite chemical status in the substrate.

TECHNIC

Eighty 2-gallon crocks of washed builders' sand were planted with eight seeds each of a pure line of Rowden 2088 cotton seed on March 22, 1937. On

April 5, all but the three most uniform seedlings in each culture were removed, and the most uniform cultures were selected to receive the 22 different nutritional treatments in triplicate. The application of nutrient solutions was started on April 8, using the constant drip technic (8) with a rate of 2 liters daily.

The nutrient solutions were so devised that the four cations K^{+}, Ca^{++}, Mg^{++}, and Fe^{+++} comprised the variables in a system characterized by a pyramidal design which allowed variation in four components while the total

TABLE 1

Proportional parts of component cations in nutrient solutions

SOLUTION NUMBER	PROPORTIONAL PARTS			
	K^{+}	Ca^{++}	Mg^{++}	Fe^{+++}
1	1	1	1	37
2	1	1	37	1
3	1	37	1	1
4	37	1	1	1
5	1	13	13	13
6	13	1	13	13
7	13	13	1	13
8	13	13	13	1
9	3	3	3	31
10	3	3	31	3
11	3	31	3	3
12	31	3	3	3
13	7	7	7	19
14	7	7	19	7
15	7	19	7	7
16	19	7	7	7
17	3	10	25	2
18	3	25	10	2
19	10	3	25	2
20	10	25	3	2
21	25	10	3	2
22	25	3	10	2

number of proportional parts remained constant. Other ions present were maintained at a constant level in all solutions. Each proportional part was taken as 0.000025 equivalent per liter of nutrient solution. This value for the proportional part was empirically chosen, but it does have the convenience of being equivalent to 1 p.p.m. of potassium or $\frac{1}{2}$ p.p.m. of calcium. It was based on the findings of Bartholomew and Janssen (1) that 1 p.p.m. of potassium in the nutrient solution allows limited growth of the cotton plant but is definitely below adequacy. All nutrient solutions contained 40×0.000025 or 0.001 equivalent per liter of the four cations mentioned. The proportional parts of these four cations in the various nutrient solutions are given in table 1.

These four cations were all added as nitrate salts, supplying, thereby, 0.001 equivalent per liter or 14 p.p.m. of nitrate nitrogen. Phosphorus was added as mono-ammonium phosphate; and sulfur, as ammonium sulfate, each at the rate of 2 p.p.m. Sufficient ammonium nitrate was added that the total nitrogen content of all nutrient solutions was 20 p.p.m., 15.5 parts being supplied as NO_3-N and 4.5 parts as NH_4-N. Boron was added as boric acid, and manganese was added as manganese sulfate, each at the rate of $\frac{1}{4}$ p.p.m. The stock solutions were made up as follows:

SALT	MOLARITY	GRAMS/LITER	
KNO_3	0.10	10.1	
$Ca(NO_3)_2 \cdot 2H_2O$	0.05	10.0	
$Mg(NO_3)_2 \cdot 6H_2O$	0.05	12.8	
$Fe(NO_3)_3 \cdot 9H_2O$	0.0333	13.4	
$(NH_4)_2SO_4$	0.0284	3.75	(All in same liter of solution)
NH_4NO_3	0.0426	3.41	
$NH_4H_2PO_4$	0.0284	3.27	
$MnSO_4$		0.203	
H_3BO_3		0.142	

The concentrations of the four stock solutions supplying the cations K^+, Ca^{++}, Mg^{++}, and Fe^{+++} were such that the number of milliliters of these solutions to be added to 4 liters of the respective nutrient solution corresponded exactly with the numbers for the proportional parts of each cation given in table 1. The other stock solution, which contained the P, S, Mn, B, and additional N, was added at the rate of 10 ml. to each 4 liters of nutrient solution in order to provide the desired concentrations.

EXPERIMENTAL OBSERVATIONS

Colorimetric pH measurements were made with Clark and Lubs indicators from time to time on the solutions as made up and as they dripped from the base of the sand cultures. The results presented in table 2 were taken on the afternoon of May 3 and are typical of the results obtained on other dates.

It is to be noted that the solutions, as made up, which were high in iron concentration, were the most acid, some being as low as pH 3.8. Yet, these same high-iron nutrient solutions were the least acid when they dripped from the base of the sand culture. It is known that a predominance of cation absorption over anion absorption (H^+ and OH^- excluded) by a plant induces an increase in acidity of the nutrient medium. The converse situation also holds. Table 2 shows the factors for the amount of increase in hydroxyl-ion activity, as these various nutrient solutions dripped through cultures of growing cotton plants. The relatively large decrease in hydrogen-ion activity, or increase in $[OH^-]$, in nutrient solutions high in iron is the most striking feature of this table. By way of contrast, the solutions which underwent the smallest de-

crease in hydrogen-ion activity were relatively high in potassium. These data indicate, then, that high iron and low potassium concentrations induce a relatively high proportion of hydrogen-ion absorption for a given amount of anion absorption; whereas, when the supply of K^+ approaches adequacy, the proportion of hydrogen-ion absorption for a given amount of anion absorption is reduced.

TABLE 2

The pH values of nutrient solutions

SOLUTION NUMBER	pH AS APPLIED	pH OF DRIP			FACTOR FOR INCREASE IN HYDROXYL-ION ACTIVITY (AV.)	PROPORTIONAL PARTS OUT OF 40 IN ORIGINAL SOLUTIONS	
		#1	#2	#3		K	Fe
1	3.8	6.8	6.7	6.8	920	1	37
2	4.6	6.7	6.6	6.6	110	1	1
3	4.6	6.6	6.6	6.6	100	1	1
4	4.6	6.4	6.5	6.4	67	37	1
5	4.1	6.8	6.8	6.8	500	1	13
6	4.3	6.6	6.8	6.7	250	13	13
7	4.2	6.7	6.6	6.6	270	13	13
8	4.6	6.4	6.5	6.4	67	13	1
9	3.8	6.8	6.8	6.8	1,000	3	31
10	4.6	6.7	6.6	6.6	110	3	3
11	4.6	6.8	6.5	6.7	110	3	3
12	4.6	6.5	6.4	6.4	67	31	3
13	4.0	6.8	6.6	6.7	490	7	19
14	4.4	6.6	6.5	6.6	140	7	7
15	4.4	6.7	6.5	6.6	150	7	7
16	4.4	6.5	6.6	6.5	130	19	7
17	4.6	6.4	6.5	6.5	73	3	2
18	4.6	6.4	6.4	6.4	63	3	2
19	4.6	6.5	6.4	6.4	67	10	2
20	4.6	6.5	6.4	6.5	73	10	2
21	4.6	6.5	6.5	6.5	79	25	2
22	4.6	6.4	6.5	6.5	73	25	2

It is of interest to note the correlation coefficients between the increases in hydroxyl-ion concentration in table 2 and the respective concentrations of potassium and ferric ions in the nutrient solution:

CHANGE IN $[OH^-]$ CORRELATED WITH	CORRELATION COEFFICIENT
Potassium	−.483
Iron	.966
K/Fe	−.432
Calcium	−.278
Magnesium	−.279

For 20 degrees of freedom, a correlation coefficient with a value of ± 0.423 is significant and one with a value of ± 0.537 is highly significant (9). It is quite

evident, therefore, that the concentration of the ferric ion was the dominant influence in the substrate upon the change in active acidity arising from differential absorption by the plant. The coefficient involving potassium concentration and the K/Fe ratio barely attained significance. The reason that it was significant at all may be explained by the definite relationship with ferric-ion concentration. When calcium- and magnesium-ion concentrations were correlated with the data for increase in hydroxyl-ion concentration, the coefficients were not significant.

TABLE 3

Green and dry weights of plants

SOLUTION NUMBER	K LEVEL	Fe LEVEL	AVERAGE GREEN WEIGHT PER PLANT	PER CENT DRY WEIGHT	AVERAGE DRY WEIGHT PER PLANT
			gm.		*gm.*
1	1	37	4.23	22.2	0.94
2	1	1	13.8	17.4	2.39
3	1	1	14.1	18.2	2.57
4	37	1	40.5	17.1	6.90
5	1	13	6.22	21.0	1.31
6	13	13	6.11	21.4	1.31
7	13	13	7.78	21.1	1.64
8	13	1	31.20	17.8	5.55
9	3	31	4.44	21.4	0.95
10	3	3	17.6	17.7	3.11
11	3	3	21.2	18.2	3.86
12	31	3	31.9	17.2	5.90
13	7	19	5.67	21.9	1.24
14	7	7	21.6	18.8	4.04
15	7	7	18.6	19.4	3.59
16	19	7	22.8	18.2	4.14
17	3	2	21.8	17.5	3.81
18	3	2	26.5	16.0	4.23
19	10	2	29.1	17.1	4.99
20	10	2	29.2	17.2	5.04
21	25	2	33.1	16.9	5.61
22	25	2	36.6	16.5	6.04
Difference required for significance		5 per cent level	2.19		0.41
		1 per cent level	2.93		0.55
Standard deviation			±1.39		±0.26

The relationship between the factors for increase in $[OH^-]$ and the data for average green weight per plant for the respective treatments (table 3) gave a correlation coefficient of -0.717, which is considerably above the requirement for high significance. Thus, there appears to be a marked inverse relationship between removal of hydrogen ions from the substrate and the growth of the plants. The fact that size of the plants was not taken into account merely makes the observation more conservative, since the smallest plants were produced in substrates in which the greatest change in $[OH^-]$ was taking place.

Table 3 presents the green and dry weight data of the plants produced under the various nutritional conditions and, also, the difference in green or dry weight per plant required for significance among the plants grown in the different treatments. Plants grown in treatments 1 and 9, high-iron treatments, were the poorest plants. The data indicate the poor growth of these plants, which developed symptoms typical of those which occur under conditions of phosphorus deficiency. The leaves were, relatively, very small; their coloration presented a bronzed appearance; and many of them had necrotic areas on their margins.

There was no significant difference in growth or appearance of the plants in treatments 2 and 3, high-magnesium and high-calcium treatments respectively. Both groups of plants made a moderate amount of growth but showed mottling on the margins of the leaves, characteristic potassium deficiency symptoms.

The plants grown in treatment 4 were the largest in the experiment. In every respect, they appeared to be "normal" and were growing vigorously. It should be reiterated that these plants were receiving only $\frac{1}{2}$ p.p.m. of Ca and $\frac{8}{10}$ p.p.m. of Mg in their nutrient solution, whereas potassium was supplied at 37 p.p.m. This gives an indication of the low concentration of calcium and magnesium necessary in the substrate for cotton plants, provided that this concentration is maintained. Also, the preponderance of potassium supply above the supplies of other cations necessary to induce optimum metabolic activity in the cotton plant is indicated by these data.

Plants in treatments 5, 6, 7, and 8 indicate the marked inhibitory effect of iron on the growth of cotton plants. There is no significant difference between plants grown in solutions 5, 6, and 7 where one-third the total equivalents of variable cations were supplied as Fe^{+++}, regardless of level of supply of other cations. In treatment 8, Fe^{+++} supplies only 1 proportional part, and other cations each supply 13 parts, but growth response more than quadruples that of the previously mentioned three treatments.

Data for the growth of plants receiving solutions 9, 10, 11, and 12 confirm those for the plants receiving the first four solutions. The 3 p.p.m. of K^+ in treatments 10 and 11, however, induced appreciably more growth than did the 1 p.p.m. of K in the corresponding treatments 2 and 3. Also, plants in treatment 12 made appreciably less growth with the 3 parts Fe^{+++} than did those given treatment 4 with 1 part Fe^{+++}.

Treatments 13, 14, 15, and 16 further substantiate the inhibitory effect of Fe^{+++} upon growth of the cotton plant. Thus, with iron supplying 7 out of a total of 40 proportional parts of cations, the proportion of the other cations had little effect on growth. By comparison with plant weights produced in other treatments, e.g. treatment 8, it is apparent that solution 16 supplied sufficient K^+ to induce considerably more growth response than was observed, but, evidently, the seemingly small amount of iron present deterred utilization of the potassium available. In general, the plant responses from these four treatments correspond to those of solutions 5, 6, 7, and 8.

In treatments 17 to 22, inclusive, the iron was maintained at a level of 2

proportional parts in all, while the proportions of other cations were varied. It is apparent that growth response increased with K^+ level, but the more interesting point is the amount of growth made by the plants supplied with only 3 p.p.m. of K. This confirms the work of Bartholomew and Janssen (1) that cotton plants have the capacity to obtain an adequate amount of potassium even when the element is supplied at relatively low concentrations, provided, of course, that the concentration is kept nearly constant.

In order to obtain a better picture of the response of the cotton plant to the cation proportions used in this study, a multiple curvilinear correlation analysis was applied to the data for green weight of plants per culture and cation levels, using the successive approximation technic of Ezekiel (3). It should be pointed out, however, that care must be taken as to the interpretations derived from these regression curves. Certain peculiarities inherent in the experimental data preclude the unmodified use of orthodox deductions. As the total of all independent variables is constant, whenever one of these variables approaches the maximum, all of the others must approach the minimum. Thus, when the supply of one of the cations was 37 proportional parts, the other three were supplied at 1 proportional part each. This contingency is anomalous to the theory of multiple regression, which is based on the assumption that values occurring on any portion of the net regression curve for a given independent variable are associated with values distributed over the entire ranges of the other independent variables. Consequently, a discussion of the curves must take into account the fact that these reflect not only the influence of level of supply of a given cation, but also the effect of cationic balance, i.e., the proportion of the total variable cationic equivalence contributed by a given cation.

The net regression curves obtained are presented in figures 1, 2, 3, and 4. The standard error of estimate of these regression curves is ± 7.34 gm., and the index of multiple correlation has a value of 0.975. Thus, the net regression curves obtained permit 95 per cent (0.975^2) of the variance in growth response to be explained by variance in cation levels.

The indexes of part correlation and part determination were calculated from the net regression curves of figures 1–4. It should be borne in mind that these indexes indicate the relationship between the dependent variable, growth, and a given independent variable after taking into account the corrections for the variance of the regression curves of the other independent variables. These indexes were as follows:

INDEPENDENT VARIABLE	INDEX OF PART CORRELATION	INDEX OF PART DETERMINATION
		per cent
K^+	0.952	90.6
Ca^{++}	0.813	66.1
Mg^{++}	0.747	55.8
Fe^{+++}	0.945	89.3

After the effects of Ca, Mg, and Fe ions as given by the respective regression curves are taken into account, 90.6 per cent of the variance observed in growth response may be ascribed to variance in the level of supply of K^+. Similar relations hold for the other three varying cations. Potassium and iron

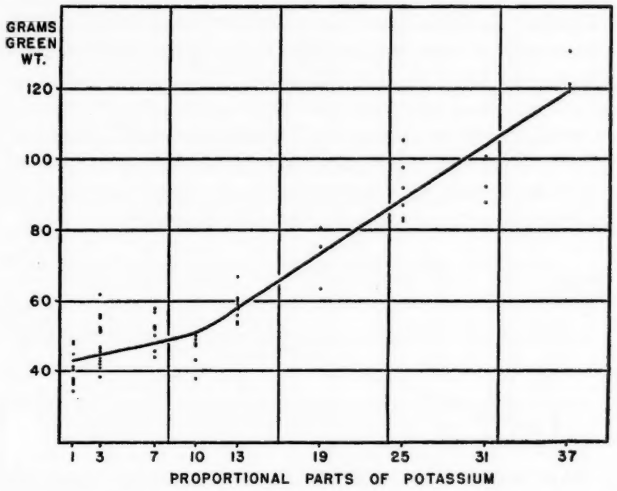

FIG. 1. RELATIONSHIP BETWEEN PROPORTIONAL PARTS OF K^+ SUPPLIED IN NUTRIENT SOLUTION AND THE GROWTH OF COTTON PLANTS, IN GRAMS OF GREEN WEIGHT

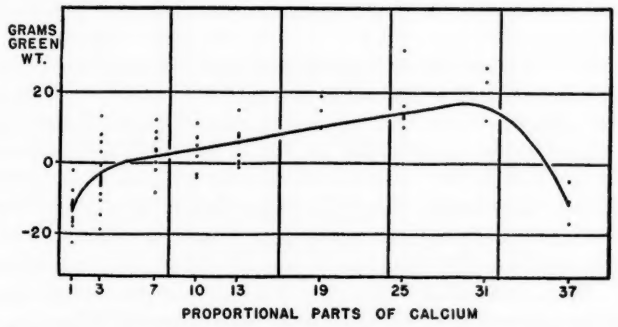

FIG. 2. ALLOWANCE, IN GRAMS GREEN WEIGHT OF COTTON PLANTS, FOR PROPORTIONAL PARTS OF Ca^{++} SUPPLIED IN NUTRIENT SOLUTION

had an appreciably greater effect on growth than did calcium and magnesium, although the influence of the last two ions was pronounced.

The net regression curve in figure 1 indicates that the relationship between growth and potassium supply was positive over the entire range studied, and

evinced the marked growth response to increments in K^+ supply. It is apparent that the optimum level of potassium supply was not attained, and that under the experimental conditions no deleterious effects were associated with the preponderance of cations being supplied as K^+.

The net regression curve of figure 2 shows that growth response to Ca^{++} was positive over nearly the entire range of Ca^{++} concentrations studied, with a distinct retroflexion in the relationship at the highest level of calcium used. This certainly does not mean that 18.5 p.p.m. of calcium in the nutrient solutions had a detrimental effect on growth as compared to 15.5 p.p.m. It is in accord with the work of Osterhout (7) upon ionic antagonism in relation to permeability and growth; that is, when the preponderance of the cations was supplied as divalent calcium, insufficient monovalent K^+ was present to counteract the injurious effect of the predominance of calcium.

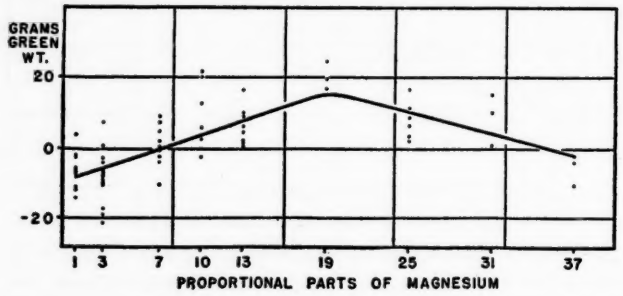

FIG. 3. ALLOWANCE, IN GRAMS GREEN WEIGHT OF COTTON PLANTS, FOR PROPORTIONAL PARTS OF Mg^{++} SUPPLIED IN NUTRIENT SOLUTION

In figure 3, the curve for the magnesium relationship shows the presence of a very definite optimum in the medial portion. Here again, it should not be interpreted that any of the concentrations of magnesium used were sufficient to depress growth *per se*; but when the higher levels of Mg^{++} were used it concomitantly follows that the concentrations of the other three cations approached their lower levels. That is, a preponderance of Mg^{++} even in such dilute concentrations introduces an unfavorable cationic balance.

The net regression curve in figure 4 presents the ferric-ion relationship to growth. The general trend of this curve is negative, with a marked minimum in its medial portion. The effect of cation balance is reflected in this curve. Certainly, increasing the iron content of the nutrient solution from 13 parts to 37 parts is not conducive to a positive growth response. But when only 13 of the proportional parts are supplied as iron, other ions may be present in sufficient amounts to enable appreciable growth were it not for the inhibitive activity of iron. When the preponderance of the cations was supplied as Fe^{+++}, the other cations were not present in sufficient quantity to have permitted appreciable growth in the absence of iron, and, hence, the Fe^{+++} had

less opportunity to effect an inhibitory influence. In other words, the positive portion of the regression curve for Fe^{+++} indicates a decrease in inhibitory effect due to a decrease in growth-inducing potentialities of the supply of other cations, and does not indicate a positive effect on growth by Fe^{+++}.

A discussion of the observed iron relation would have to take into account the results of Mattson's (5) catephoretic studies on ferric phosphate, which he found to be isoelectric at pH 4.6. The pH range of the nutrient solutions used was such that the higher concentrations of Fe^{+++} would completely precipitate the PO_4^{---} and cause phosphorus deficiency. As noted earlier, plants receiving high amounts of iron showed symptoms typical of phosphorus deficiency. It is possible that the Hardy-Shulze rule has a bearing upon the relationships found herein. By this rule, the trivalent Fe^{+++} should have a much higher coagulating effect upon the cytoplasmic membranes of the

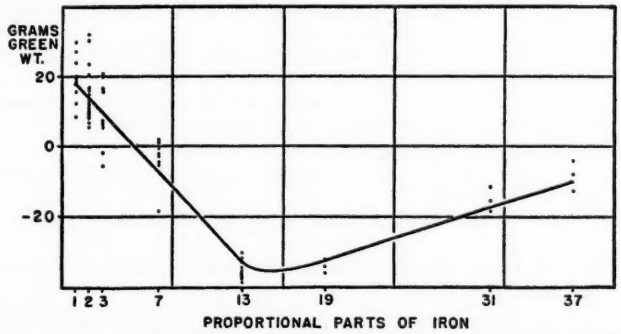

FIG. 4. ALLOWANCE, IN GRAMS GREEN WEIGHT OF COTTON PLANTS, FOR PROPORTIONAL PARTS OF Fe^{+++} SUPPLIED IN NUTRIENT SOLUTION

absorbing roots than the divalent Mg^{++} and Ca^{++}. The last two should have a much greater coagulating effect than monovalent K. A study of table 3 will show that the growth of these cotton plants was inversely related to the charge on the predominating cation. Thus, even though a major portion of this relation can be ascribed to the important nutritional role of K^+, it does seem that the electrokinetics of membrane phenomena cannot be ignored.

SUMMARY AND CONCLUSIONS

Cotton plants were grown in nutrient solutions varied as to the proportions of potassium, calcium, magnesium, and iron. These nutrient solutions were distinct in that they were relatively very dilute in concentration, only cations entered into the variability, and proportions were calculated on the equivalence basis.

Growth was directly related to level of potassium supply. Increasing the Fe^{+++} supply had a distinctly adverse effect on growth. The influence of

Ca^{++} and Mg^{++} was not so pronounced as that of the other two cations studied. Calcium and magnesium tended to be beneficial unless they supplied a preponderance of the total variable cations.

The pH changes in the substrate were inversely related to the K/Fe ratio; the higher this ratio, the less change in H^+ in the nutrient medium. It was suggested that at the higher levels of Fe^{+++} supply, a larger proportion of cations absorbed, in relation to a given amount of anion absorption, is H^+.

Of the many theoretical implications of the observed relationships, a few are discussed.

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GROWTH OF CUNNINGHAMELLA BLAKESLEEANA AS INFLUENCED BY FORMS OF NITROGEN AND PHOSPHORUS UNDER VARYING CONDITIONS[1]

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The relative growth of the fungus *Cunninghamella* on properly controlled soil cultures has recently been proposed as a measure of the level of available phosphorus in soils (14, 15). Because the method offers considerable promise, a study has been made of the nutrition of this fungus, particularly as regards the suitability of different forms of nitrogen and phosphorus. The results of the investigation are reported in this paper.

Very little specific information on the nutrition of *Cunninghamella* was found in the literature. Previous studies have been restricted largely to morphologic and genetic classification. The forms of nitrogen which various fungi preferably utilize have been widely investigated; for a general review of the literature the reader is referred to the texts of Löhnis (12), Waksman (27), and Lafar (10), and to the papers of Klotz (9), and Janke (8). Whether the ammonium, nitrate, amide, or amino forms of nitrogen are used at all, or with preference, appears to depend upon the fungus, the reaction, the carbon source of the culture medium, and the isoelectric point (17). *Cunninghamella bertholletia* at a slightly acid reaction was able to use the nitrate form rather well (23). In the presence of 1 per cent citric acid and 10 per cent sucrose, *Cunninghamella* sp. apparently was unable to develop with ammonium sulfate as the source of nitrogen (22). *Cunninghamella elegans* and *Cunninghamella echinulata* used the ammonium and nitrate forms equally well at neutral and slightly acid reactions (14).

The usual forms of phosphorus which are supplied to fungi are the salts of orthophosphoric acid, although the meta- and pyro- forms of phosphoric acid and organic forms have been used (3). For a review of the literature on the forms of phosphorus and the extent of their utilization by fungi, the reader is referred to Löhnis (12) and Waksman (27). The assimilation of water-

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insoluble or slightly soluble forms of phosphorus by fungi is closely related to the formation of acid in the metabolic processes of the fungi and partly to the action of enzymes. In the assimilation of phosphorus, reduction may also play a role (18). The acidity of the culture medium influences considerably the assimilation of phosphates by *Aspergillus niger* (25). *Rhizopus* sp. utilized phosphorus from the soil approximately in proportion to the phosphorus soluble in 1 per cent citric acid (21). *Cunninghamella elegans* and *Cunninghamella echinulata* grown in soil cultures near the neutral point developed in proportion to the solubility of the applied phosphates (14).

MATERIALS AND METHODS

The method followed in general in the present work was that of Mehlich, Fred, and Truog (14), in which a solid substratum contained in a small cavity of a special clay dish or slab (15) is used, and the extent of growth of the aerial development of the fungus mycelium (diameter of the colony) is measured directly. *Cunninghamella blakesleeana* (minus strain) was employed (11).

Sand and soil cultures

Optimum conditions for the growth of the fungus in sand cultures were provided by mixing two parts of quartz flour with one part of washed quartz sand. This mixture, from the standpoint of texture, serves as a very satisfactory substratum for growth. Powdered bentonite variously saturated was employed to buffer this sand at different reactions. The use of 6 per cent of natural bentonite containing some free $CaCO_3$ buffered the system at pH 8.6; a combination of 5 per cent of a 96 per cent K-saturated bentonite and 1 per cent of the natural bentonite buffered it at pH 7.5; and 6 per cent of a 51 per cent K-saturated bentonite buffered it at pH 6.7. By means of H-saturated bentonite, systems at pH 5.3 and pH 2.9 were obtained. The latter was made possible by treating the sand and bentonite mixture with dilute citric acid and washing with distilled water to remove the excess of acid. Systems of intermediate points of reaction were compounded of mixtures of the systems just described. The K- and H-bentonites were prepared by Tyner (26) and Cook (4) respectively. For the determination of pH, the glass electrode and Truog's triplex indicator method were used.

For soil cultures, air-dry surface soil powdered to pass a 20-mesh sieve was used.

Forms of nitrogen used

The inorganic salts of nitrogen, asparagin, aspartic acid, glycine, cystine, and urea used were commercial, chemically pure products; the glutamic acid and arginine were laboratory preparations. The fertilizers were commercial products. The mold tissues were obtained from nutrient liquid cultures. They were washed free of the inorganic salts, and ground to pass a 60-mesh sieve. Alfalfa hay was similarly treated.

Forms of phosphorus used

With the exception of the crude phytin, which was a laboratory preparation, and metaphosphate, which was prepared at Muscle Shoals, all inorganic and organic phosphorus salts used were commercial, chemically pure products. Before being used, the iron and aluminum phosphates were washed to remove the water-soluble portion. The basic iron phosphate—dufrenite—was an impure sample containing 19.2 per cent P_2O_5; and before being used it was treated with CO_2-saturated water to remove any easily soluble phosphorus. The rock phosphate was a 200-mesh commercial product.

Nutrient solution

For convenience, a stock solution of inorganic salts having the following composition was prepared:

K_2SO_4	10.00 gm.
$FeSO_4 \cdot 7H_2O$	0.05 gm.
$MgSO_4 \cdot 7H_2O$	5.00 gm.
$ZnSO_4 \cdot 7H_2O$	0.05 gm.
Distilled water	100 cc.

The final nutrient solution was made up fresh each time just before use. For the test with forms of phosphorus, it was prepared as follows:

Sucrose	2.5 gm.
Asparagin	0.5 gm.
l-Aspartic acid	0.1 gm.
Stock solution of inorganic salts	0.5 cc.
Distilled water	100 cc.

For the test with forms of nitrogen, asparagin and *l*-aspartic acid were omitted, and 0.1 gm. of $CaH_4(PO_4)_2 \cdot H_2O$ was added in all cases.

Addition of different forms of nitrogen and phosphorus

The soluble forms of nitrogen and phosphorus tested were added in the desired concentration to the nutrient solution, of which a definite volume, usually 2 cc., was then added to 10 gm. of sand or soil. The plaque was brought to its optimum moisture content by adding some nutrient solution free of nitrogen or phosphorus. To obtain a satisfactory distribution of the water-insoluble forms of nitrogen and phosphorus with substratum (sand or soil), the required amount of material was first mixed thoroughly in a mortar with a small amount of fine quartz sand, after which this portion was thoroughly mixed with the soil or sand.

INFLUENCE OF FORMS OF NITROGEN AND REACTION ON GROWTH

It was found previously (14) that, within a certain narrow range of reaction, both the ammonium and the nitrate forms of nitrogen are used by *Cunning-*

hamella. In the present study, organic forms of nitrogen were included, and the range of reaction was extended.

After the concentration of soluble nitrogen necessary to obtain maximum growth of the fungus was determined, different forms of nitrogen were added at a rate to provide 5 mgm. of nitrogen per 100 gm. of sand-bentonite mixtures. The results thus obtained are presented graphically in figure 1.

Cunninghamella blakesleeana is apparently able to use the nitrate form equally well throughout a wide range of reaction, whereas the ammonium form

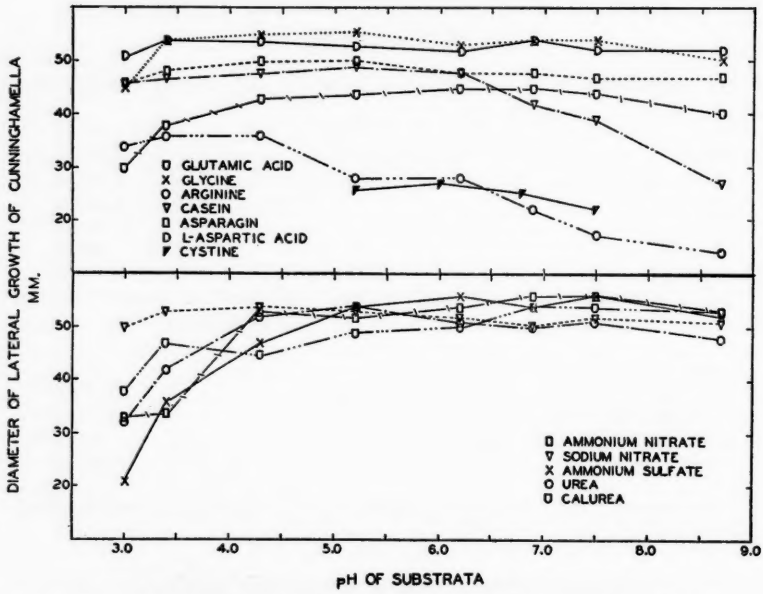

FIG. 1. GROWTH OF CUNNINGHAMELLA BLAKESLEEANA IN SAND CULTURES WITH DIFFERENT FORMS OF NITROGEN AND AT DIFFERENT REACTIONS

Rate of application, 5 mgm. N per 100 gm. sand, except cystine, which was applied at the rate of 20 mgm. N per 100 gm. sand.

is used equally well only above pH 5 and to a considerably less extent below pH 4. That ammonium nitrate is not used so well at a reaction more acid than pH 4 is undoubtedly due to the less favorable action of the portion existing as ammonium. It is interesting to note that urea and calurea (containing about one-fifth of its total nitrogen in the nitrate form), both of which yield ammonium on decomposition, influenced growth much as did ammonium sulfate. That reaction plays an important role in the assimilation of different forms of nitrogen by fungi has been clearly demonstrated by Rippel (17), Sakamura (19), and others (12, 27).

Of the monoamino-monocarboxylic acids used, the glycine was utilized equally well at all reactions. Cystine applied at the rate of 20 mgm. N per 100 gm. sand was assimilated only to a slight extent. Of the monoamino-dicarboxylic acids used, aspartic acid permitted maximum growth throughout the range of pH 3.0 to 8.6, whereas the glutamic acid, though not permitting maximum growth, was utilized equally well at all reactions above pH 4. The diamino-monocarboxylic acid, arginine, was utilized to a moderate degree on the acid side and least on the alkaline side. The acid amide, asparagin, was assimilated equally well at all reactions. The casein, containing a variety of amino acids, was utilized best on the acid side.

To determine to what extent *Cunninghamella blakesleeana* can grow in the presence of more complex nitrogenous materials, a number of organic fertilizers, calcium cyanamid, mold, and plant tissues were added to sand-bentonite mixtures. The results obtained are given in table 1. The best growth was ob-

TABLE 1

Relative growth of Cunninghamella *on cyanamid and organic forms of nitrogen added on the basis of 20 mgm. nitrogen per 100 gm. sand*

REACTION	DIAMETER OF LATERAL GROWTH WITH DIFFERENT NITROGEN SOURCES								
	Calcium Cyanamid	Cotton-seed Meal	Tankage	Dried Blood Meal	Milor-ganite	Nettolin	Mold Tissue (*A. niger*)	Mold Tissue (*A. sydowy.*)	Alfalfa Hay
pH	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
5.2	12	45	31	29	40	54	44	53	31
6.0	18	50	26	27	37	54	46	54	34
6.8	17	47	30	31	40	53	40	50	33
7.5	12	48	19	30	40	50	39	50	33

tained with Nettolin (processed peat), which may be due partially to its content of a small amount of nitrate nitrogen. Cottonseed meal, Milorganite (activated sludge product), and mold tissue produced growths approaching that of Nettolin. With calcium cyanamid, no growth was obtained; but with a second sample kept moist for 14 days and then inoculated with spores of *C. blakesleeana*, growth took place as shown. That the fungus can utilize nitrogen from mold tissue and alfalfa hay is also indicated.

The results obtained, in general, would lend support to the idea that *Cunninghamella blakesleeana* might be used for measuring the nitrogen deficiencies of soil. The fungus not only responded to the forms of nitrogen available to plants, namely, nitrate and ammonium, but also to other forms of nitrogen which are considered potential sources for plants.

INFLUENCE OF FORMS OF PHOSPHORUS AND RELATED CONDITIONS ON GROWTH

To study the influence of different forms of phosphorus on the growth of *Cunninghamella blakesleeana*, sand-bentonite mixtures and soils were used as

the substrata. The influence of pH, iron and aluminum oxides, and calcium carbonate on the availability of phosphorus to this fungus was also investigated.

The influence of forms of phosphorus on growth in relation to reaction in sand cultures is shown in figure 2. Of the inorganic phosphates, the mono- and dicalcium phosphates and magnesium phosphate are available throughout

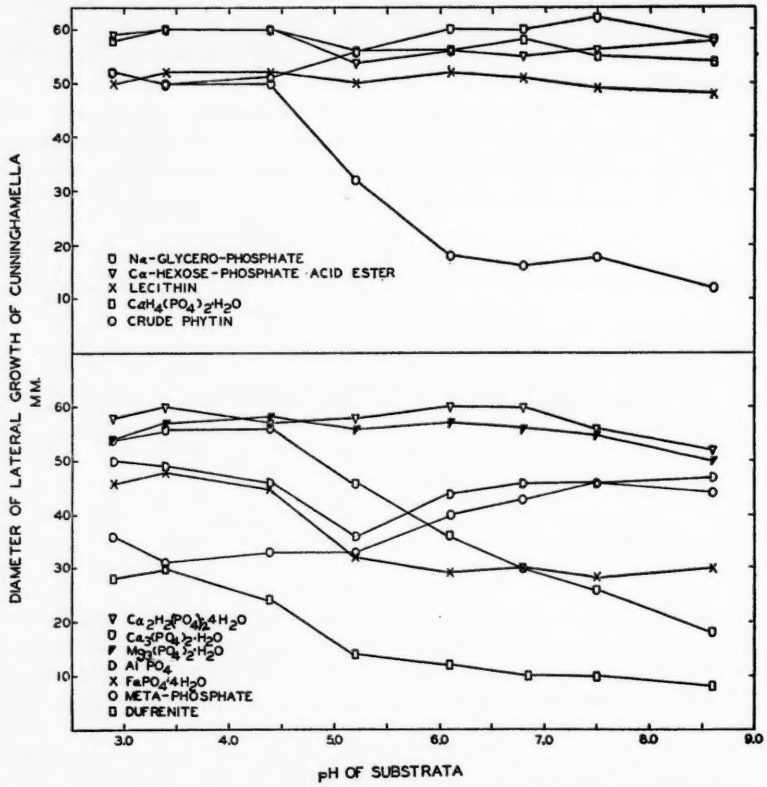

FIG. 2. GROWTH OF CUNNINGHAMELLA BLAKESLEEANA IN SAND CULTURES WITH DIFFERENT FORMS OF PHOSPHORUS AND AT DIFFERENT REACTIONS

Rate of application, 5 mgm. P_2O_5 per 100 gm. sand except dufrenite and crude phytin, which were applied at the rate of 20 mgm. P_2O_5 per 100 gm. sand.

a wide range of reaction. Above pH 8 the dicalcium phosphate and the magnesium phosphate produced less growth. The tricalcium phosphate is readily available on the acid side and becomes gradually less available toward the neutral and alkaline side. *Cunninghamella blakesleeana* can use to an appreciable extent the aluminum and iron phosphates and the metaphosphate. Aluminum phosphate and the metaphosphate are absorbed on the alkaline

side better than tricalcium phosphate. Some investigators obtained similar results (1, 13, 24) with plants. That plants can use the metaphosphate has been shown by several investigators (6, 28). With the exception of phytin, which is utilized somewhat better on the acid side, the organic phosphates are readily available throughout the range of reaction studied.

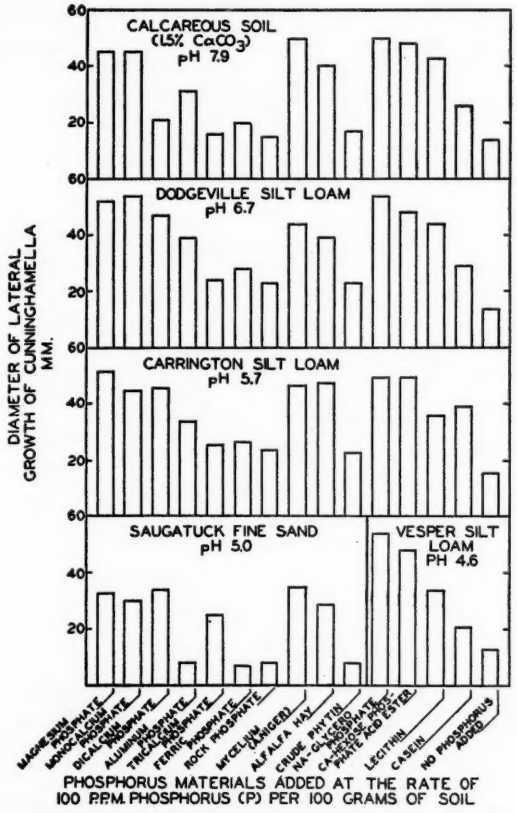

FIG. 3. GROWTH OF CUNNINGHAMELLA BLAKESLEEANA IN SOIL CULTURES WITH DIFFERENT FORMS OF PHOSPHORUS

Figure 3 shows the influence of forms of phosphorus on growth of *Cunninghamella blakesleeana* in soil cultures. The different forms are utilized largely in proportion to their solubility. The presence of free calcium carbonate in a soil renders the tricalcium phosphate appreciably less available and the dicalcium phosphate somewhat less available. Other phosphates, notably the organic forms of phosphorus, are considerably less affected by the calcium carbonate. Saugatuck fine sand has a phosphorus fixing capacity of 80 per

cent, as determined by the method of Heck (7). As a result of this high fixing capacity, the availability of the soluble phosphates applied to this soil for fungus growth was considerably reduced, iron and aluminum phosphates and phytin being used but little.

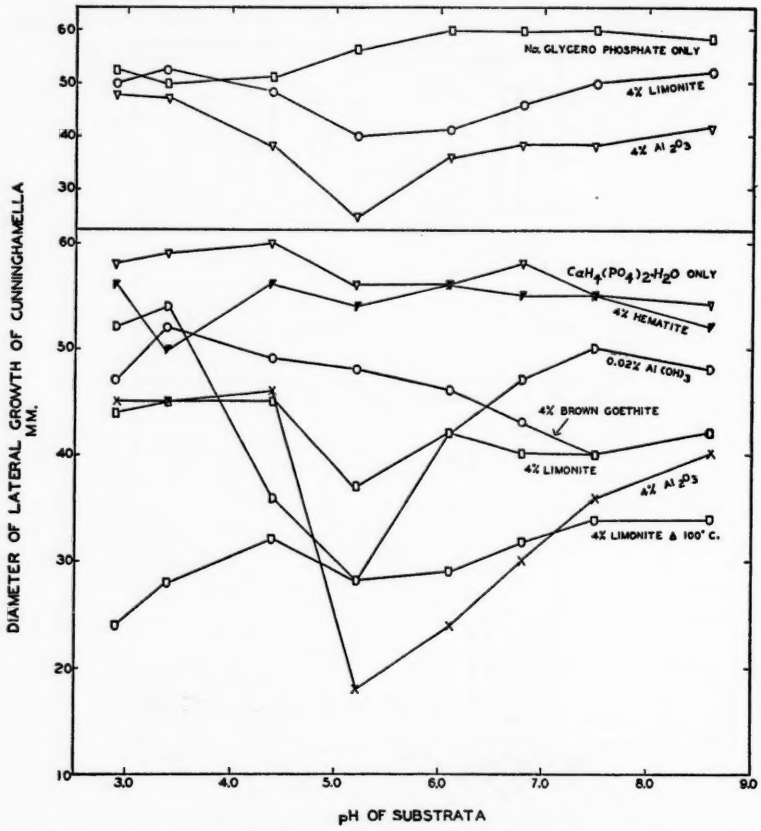

FIG. 4. GROWTH OF CUNNINGHAMELLA BLAKESLEEANA IN SAND CULTURES AS INFLUENCED BY IRON AND ALUMINUM OXIDES AT DIFFERENT REACTIONS

Monocalcium phosphate and sodium glycerophosphate were applied alone and with additions indicated at a rate to give 5 mgm. P_2O_5 per 100 gm. sand.

Influence of iron and aluminum oxides on availability of different forms of phosphorus

The extent to which the fungus can use the different forms of phosphorus appears to depend mainly on the solubility of these forms and upon the factors which influence the solubility during the growth of *Cunninghamella blakes-*

leeana. To observe how iron and aluminum oxides influence the availability of a soluble phosphate, hematite, goethite, limonite, aluminum oxide, aluminum hydroxide, and diorite were added to sand-bentonite mixtures containing 5 mgm. P_2O_5 per 100 gm. as monocalcium phosphate. The results, given in figure 4, indicate that the growth of *Cunninghamella blakesleeana* was un-

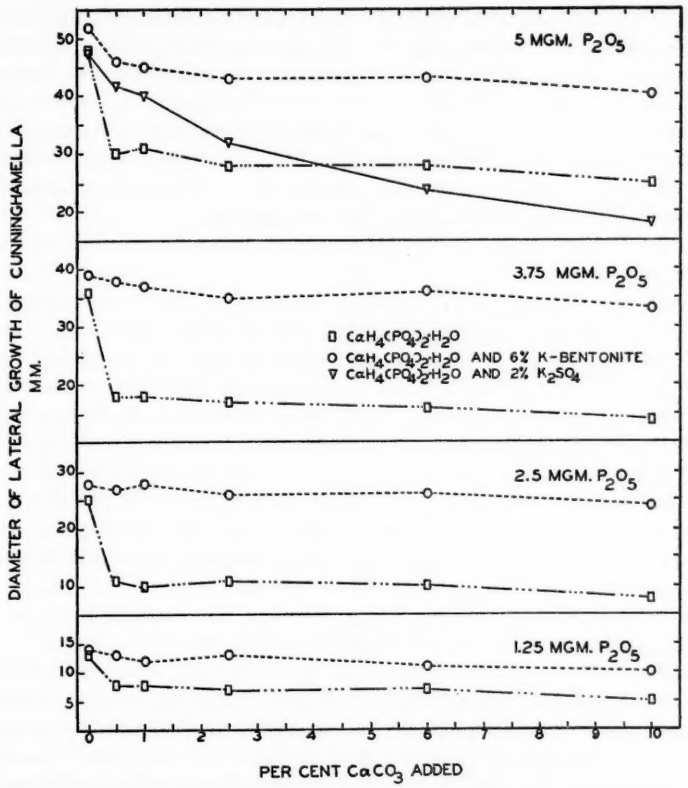

FIG. 5. GROWTH OF CUNNINGHAMELLA BLAKESLEEANA IN SAND CULTURES WITH INCREASING CONCENTRATIONS OF P_2O_5, AND CALCIUM CARBONATE AND WITH THE ADDITION OF BENTONITE AND POTASSIUM SULFATE

affected by hematite but was slightly reduced by goethite and significantly reduced by limonite, especially when the soluble phosphate was previously dried in contact with the limonite for 12 hours at 100°C. The aluminum oxide was particularly effective in rendering the phosphorus difficultly available for growth at pH 5.2 and pH 6.1. Colloidal $Al(OH)_3$ (precipitated), added in concentrations of only 0.02 per cent, markedly reduced the availability of

monocalcium phosphate at pH 5.2. In the presence of limonite and aluminum oxide, the availability of sodium-glycerophosphate was also appreciably reduced.

Influence of calcium carbonate on the availability of phosphorus

When to a sand culture containing phosphate but not bentonite, increasing amounts of calcium carbonate were added, growth of *Cunninghamella blakesleeana* was greatly reduced (fig. 5). The presence of 0.5 per cent of calcium carbonate reduced growth nearly 50 per cent. On adding 6 per cent of a 96 per cent K-saturated bentonite, the efficiency of calcium carbonate in reducing the growth of the fungus became appreciably less at all concentrations. The influence of potassium sulfate was similar to that of bentonite when the calcium carbonate concentration was small. At the higher concentrations of calcium carbonate, the soluble salt tended to decrease growth.

DISCUSSION

Under carefully controlled growing conditions of optimum temperature, humidity, and aeration, the growth of *Cunninghamella blakesleeana* can be closely duplicated, and with careful measurements, variations will rarely exceed 10 per cent. This is based on the results of numerous determinations which gave an average deviation of 3 mm. in diameter. In some cases the analysis of variance was used to check the conclusion as drawn from the curves.

The use of different bentonite systems has made it possible to buffer sand cultures sufficiently to prevent any significant changes in reaction due to the addition of different forms of nitrogen and phosphorus. Although the addition of bentonite to systems may have other than buffering influences, serious errors from these influences are not to be expected.

Cunninghamella blakesleeana will grow over a wide range of reaction—pH 2.9 to 8.7 (good growth was obtained on a soil of pH 9.6)—and is not easily influenced directly by acid or alkali, yet it is very sensitive to different forms and concentrations of phosphorus and nitrogen. Ammonium salts and organic compounds like urea, which on decomposition yield ammonium, promote significantly less growth in an acid medium than do nitrate salts. The amino acids promoted the least growth near their isoelectric points, which are for arginine pH 10.8, for glutamic acid pH 3.2, for aspartic acid pH 2.8, and for glycine pH 6.0. This effect is essentially due to the lower solubility of the amino acids near their isoelectric points.

Although *Cunninghamella blakesleeana* grows best on water-soluble forms of organic and inorganic phosphorus, it will grow on some forms of water-insoluble phosphorus, probably because of production of acid in its metabolic processes. The substratum usually becomes slightly more acid below the center of growth than in the surrounding area. Thus, the rate of growth in the presence of soluble phosphorus is rapid from the beginning, but in the presence of water-insoluble forms, it is slow at first, but becomes rapid, usually after 30 hours of incubation.

Factors which influence the solubility of phosphorous will indirectly influence the growth of the fungus. Thus in an acid medium, many water-insoluble phosphates will be readily utilized. The presence of hydrated iron or aluminum oxides greatly reduces the solubility of phosphorus and hence retards growth. This is particularly marked at reactions where insoluble aluminum or iron phosphates are formed. Large amounts of iron or aluminum oxides may influence the availability of phosphorus not only at very acid, but also at moderately basic reactions. Very small amounts of fresh, colloidal $Al(OH)_3$ decrease the solubility of phosphorus, particularly at pH 5.2 and somewhat less at pH 6.0.

Calcium carbonate reduces the availability of many phosphates to *Cunninghamella blakesleeana*. The effect of calcium carbonate is modified in the presence of a soluble salt or an exchange material containing a monovalent cation. Relatively large amounts of a soluble salt, like K_2SO_4, at the lower concentrations of calcium carbonate promote growth; at higher concentrations of calcium carbonate this influence was not observed. In the presence of exchange material, the soluble calcium arising from calcium carbonate is removed from the immediate sphere of growth, being replaced by the monovalent cation of the exchange material, and consequently, even at the higher concentrations of calcium carbonate, growth is not significantly reduced.

Although Pierre found that higher plants cannot use organic phosphorus directly (16), other invesitgators obtained good yields with organic forms of phosphorus that were readily mineralized by microorganisms (2, 20). Phytin can be used by plants largely in proportion to biological activity in which the catalytic action of phytase apparently plays an important part (5). It is significant to note that *Cunninghamella blakesleeana* can use to an appreciable extent only those forms of organic phosphorus which are readily mineralized.

SUMMARY

An investigation of the nutrition of *Cunninghamella blakesleeana* (minus strain), particularly as regards the suitability of different forms of nitrogen and phosphorus under various conditions, was undertaken in both sand and soil cultures. Sand cultures were buffered at different pH values by means of hydrogen- and potassium-saturated bentonites.

The nitrate form of nitrogen promoted the growth of *Cunninghamella blakesleeana* equally well over a pH range of 2.9 to 8.6. The ammonium form, urea, and calurea produced good growth above pH 5 but appreciably less growth with increasing acidity. The monoamino acids, glycine and aspartic acid, gave a maximum growth over a wide range of reaction. Arginine and glutamic acid tended to promote least growth near the pH values of their isoelectric points. Cystine was found to be a poor source of nitrogen for *Cunninghamella blakesleeana*. The fungus was able to make fairly good growth on some complex nitrogenous materials and organic fertilizers which probably contained easily mineralized nitrogen.

Water-soluble phosphates promoted growth equally well over a wide range of reaction, pH 2.9 to pH 8.6. The utilization of slightly soluble or water-insoluble phosphates varied with reaction, and fairly good growth was obtained on some forms of water-insoluble phosphates. Organic forms of phosphorus were utilized largely in proportion to the ease with which they can be mineralized.

Growth in the presence of water-soluble phosphate was reduced over a wide range of reaction in the presence of 4 per cent limonite, goethite, and aluminum oxide. Freshly precipitated aluminum hydroxide reduced growth markedly at a slightly acid reaction. Calcium carbonate in considerable amounts reduced growth significantly, but at lower concentrations this effect was diminished, especially in the presence of potassium bentonite or a soluble salt like K_2SO_4.

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AUXIN PRODUCTION BY SOIL MICROORGANISMS

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Nielsen (13) was probably the first to recognize that microorganisms produce plant growth hormones. Prior to his work investigators had isolated 3-indol acetic acid and 3-indol proprionic acid from bacterial fermentations (5, 17, 18), but these compounds were not recognized as auxins. Since the publication of Nielsen's work, several authors have noted the production of auxins by certain yeasts, molds, and bacteria. Table 1 gives a digest of the literature.

TABLE 1

Digest of the literature reporting auxin production by microorganisms

ORGANISM	AUXIN	AUTHOR
Mixed culture	3-indol acetic acid	Salkowski and Salkowski (18). 1880
Mixed culture	3-indol proprionic acid	Salkowski (17). 1899
E. coli	3-indol acetic acid	Hopkins and Cole (7). 1903
Proteus vulgaris	3-indol acetic acid	Herter and Ten Brock (6). 1909
B. mycoides, several cocci, and members of colon-typhoid dysentery group	3-indol acetic acid	Frieber (5). 1921
Rhizopus suinus and *Absidia ramosa*	Not identified	Nielsen (13). 1930
Aspergillus niger	Not identified	Boysen-Jensen (1). 1931
16 species of bacteria	Not identified	Boysen-Jensen (2). 1931
Brewer's yeast	Not identified	Nielsen (14). 1931
Boletus edulis	Not identified	Nielsen (15). 1932
Yeast	3-indol acetic acid	Kögl and Kostermans (12). 1934
Rhizopus suinus	3-indol acetic acid	Thimann (19). 1935
Six species of molds	3-indol acetic acid	Ronsdorf (16). 1936
Ph. tumefaciens	Not identified	Brown and Gardner (3). 1936
Root nodule bacteria	Not identified	Thimann (20). 1936

In a series of papers published between 1933 and 1935, Kögl and his co-workers (8, 9, 10) determined the chemical constitution of two plant hormones which they called auxins *a* and *b*. As a result of studies with these auxins, a third auxin, called *hetero-auxin*, was discovered in urine. Hetero-auxin was shown to be identical with 3-indol acetic acid (11). Since bacteriologists had

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known for many years that some microorganisms produce 3-indol acetic acid by oxidative deaminization of tryptophane, the identification of 3-indol acetic acid as an auxin made it quite apparent, in 1934, that the production of auxins by microorganisms is not uncommon.

Though it seems quite probable that a considerable number of species of microorganisms elaborate auxins, few species have actually been investigated. So far as we are aware, almost nothing is known of auxin production by those species of microorganisms numerically predominant in soil. Nevertheless, suggestions have been made that certain little-understood plant responses may be due to growth hormones produced in the soil by the action of microorganisms on organic matter. That auxin production in soil and at root surfaces might be of considerable significance to the plant can hardly be questioned.

Thus a need is indicated for a somewhat comprehensive survey of soil microorganisms to determine how commonly these are potential auxin producers. The present report is concerned with such a survey. Various attempts have been made to determine whether auxins are produced in soil under different conditions, and some attention has been given to the significance of auxins produced in soil, but these studies are incomplete and are not included in the present report.

METHODS

The cultures employed in this study were obtained from various Indiana soils. The bacteria and actinomycetes were isolated from dilution plates of sodium albuminate agar; and the molds, from peptone-glucose-acid agar. Microscopic examinations were made to insure the purity of each culture before it was used. No attempt was made to identify the various cultures.

The media used for cultivation of the microorganisms for auxin assays were beef extract-peptone agar and a synthetic medium containing a Frazier-Rupp (4) mineral base with glucose, asparagine, and ammonium sulfate. Duplicate plates of each medium were prepared for each culture. The plates were inoculated by streaking and were incubated at 30°C. under aerobic conditions. The beef extract-peptone agar plates were incubated for 12 days before being tested, and the plates containing the synthetic medium were incubated for 30 days before being tested. Solid media were preferred to liquid media because little auxin was produced in liquid media.

Auxin determinations were made by the *Avena* test essentially as recommended by Went and Thimann (21, p. 29) for water culture. Agar blocks approximately 10 cmm. in volume were cut from the sterile agar immediately adjacent to the streaked culture and placed on *Avena* coleoptiles maintained at a temperature of 24°C. and at a humidity near saturation. Two auxin determinations were made on each of the duplicate plate cultures on each medium. The negative curvatures of the coleoptiles were determined by the use of a protractor such as that described by Went and Thimann (21, p. 33). No attempts were made to identify the auxins produced.

RESULTS

Auxin determinations, the results of which are presented in tables 2 and 3, are expressed as plant units contained in the 10-cmm. agar test block. According to Went and Thimann (21), a plant unit is that amount of auxin which, when applied in a 10-cmm. agar block to an *Avena* coleoptile, will

TABLE 2

Production of auxin from beef extract-peptone agar by various groups of soil microorganisms

ORGANISMS TESTED	NUMBER OF SPECIES TESTED	FREQUENCY DISTRIBUTION IN PERCENTAGE OF SPECIES TESTED PRODUCING INDICATED PLANT UNITS (P.U.) OF AUXIN*								AVER. P.U. PRODUCED (ALL CULTURES)	AVER. P.U. PRODUCED (POSITIVE CULTURES ONLY)
		0 p.u.	1–5 p.u.	6–10 p.u.	11–15 p.u.	16–20 p.u.	21–25 p.u.	26–30 p.u.	31–35 p.u.		
Bacteria											
Gram-negative rods	24	21	4	4	17	17	21	4	12	16	20
Gram-positive rods	37	27	5	5	13	16	13	11	8	14	19
Cocci	12	16	0	9	9	24	24	9	9	18	21
Total bacteria	73	23	4	5	14	18	18	8	10	15	20
Molds	39	54	0	11	20	8	5	3	0	6	14
Actinomycetes	38	34	3	6	16	18	21	3	0	11	18

* Plant units of auxin produced in a 10-cmm. agar test block.

TABLE 3

*Auxin production from mineral salts-asparagine-glucose-ammonium sulfate agar by various groups of soil microorganisms**

ORGANISM	P.U.†	ORGANISM	P.U.
Gram-positive R55	26	Gram-negative 54	21
40	14	11	31
R58	28	65	29
25	32	67a	18
16	15	45	16
30	10	216a	18
R69	20	44	18
33	16	Coccus 52a	17
58a	25	Actinomycetes 404	8
37	16	415	24
13	20		
53	9		

* Negative cultures omitted from the table.

† Plant units of auxin in a 10-cmm. agar test block.

cause 1° of curvature. Hence our figures representing plant units of auxin also represent the degrees of coleoptile curvature obtained in each case.

The results obtained by testing 150 species of Gram-positive and Gram-negative rods, cocci, molds, and actinomycetes on beef extract-peptone agar are presented in table 2. Ninety-nine, or approximately 66 per cent of the cul-

tures studied, were found to produce auxin from the substrate used. Seventy-seven per cent of the bacteria, 66 per cent of the actinomycetes, and 46 per cent of the molds tested were positive auxin producers, the average number of plant units produced by these positive bacteria, actinomycetes, and molds being 20, 18, and 14 respectively. Seventy-nine per cent of the Gram-negative rods, 73 per cent of the Gram-positive rods, and 83 per cent of the cocci produce auxin in approximately equal average concentrations.

One hundred cultures were streaked on the synthetic medium previously mentioned, but only 75 grew sufficiently well to warrant their being tested. Of these 75 species, 23 were Gram-positive rods, 11 Gram-negative rods, 4 cocci, 14 molds, and 23 actinomycetes. The species which were found capable of synthesizing auxin from the constituents of the synthetic medium are listed in table 3. It will be noted that 12 Gram-positive rods, 7 Gram-negative rods, 1 coccus, and 2 actinomycetes were able to synthesize auxin on the medium used. None of the molds tested is able to produce auxin from the synthetic medium. With one exception, all of the cultures which were able to produce auxin on the synthetic medium were also able to produce auxin from beef extract-peptone agar.

It might be expected that microorganisms would produce 3-indol acetic acid either as an intermediate or as an end product of the catabolism of tryptophane. Hence, the production of auxin by the action of microorganisms on organic materials containing tryptophane is expected. On the basis of known essential physiology of microorganisms, it would seem to be less expected that so high a proportion of the organisms should synthesize auxin from a relatively simple medium without tryptophane. The auxin appears actually to be synthesized and not to be the result of autolytic processes, since auxin has been detected in cultures in which almost all the cells subsequently sporulated, leaving little visible cell debris. The synthesis of auxins by numerous microorganisms, especially bacteria, strongly suggests that auxin may have a function in bacterial physiology. Presumably, some species of molds, bacteria, and actinomycetes do not produce auxins, although this is by no means certain. It is possible that under proper conditions and with sufficiently delicate experimental methods, some, if not all, of our negative cultures might be found to produce detectable concentrations of auxin.

Our results indicate that the soil flora is potentially capable of producing appreciable amounts of auxin in soils or elsewhere in nature where sufficient minerals or organic materials are present to support microbial growth.

SUMMARY

One hundred and fifty species of actinomycetes, bacteria, and molds from Indiana soils have been tested to determine their capacity to produce plant growth hormones from beef extract-peptone agar. Seventy-five of these species were also tested for auxin production from a synthetic medium without tryptophane. Sixty-six per cent of the species produced auxin on the organic medium; and 30 per cent, from the constituents of the synthetic medium.

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A REVISED METHOD FOR THE MICROSCOPIC EXAMINATION OF NATURAL STRUCTURE AND PORE SPACE IN SOILS

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Information concerning the natural structure and pore space in undisturbed samples of soil has been limited, because methods for making a microscopic examination of the soil profile were not devised until recently for this type of research.

Ross (4) has recommended a method for the preparation of thin sections of porous rocks by impregnation with Bakelite varnish diluted with a mixture of ether and methyl alcohol. The samples are submerged in a dilute solution of varnish to fill the pores and are dried at a temperature between 70° and 100°C. to remove the solvent and polymerize the varnish to form Bakelite. He also prepared sedimentary materials for microscopic study (5) by impregnation with Kollolith, which is a satisfactory cementing material because it can be melted easily, bubbles do not form when it solidifies, and when heated to 170°C. for several hours it remains clear and colorless. Kollolith is tougher than Canada balsam and can be readily polished with fine emery paper. Kubiena (2, p. 70–76) has also used this method in soil research. Leggette (3) found that a solution of Bakelite varnish and methyl alcohol containing a small amount of acetone will penetrate friable rock more rapidly than will a solution of Bakelite varnish in ether and alcohol. After a specimen is treated with the fixing solution, the solvent is volatilized by placing the specimen in a container under reduced pressure in order to hasten the rate of drying. Sideri (6) does not recommend the use of any cementing material for fine-textured soils which are compact and free from sand grains. Fir resin dissolved in xylene is used for friable samples. Harper and Volk (1) used lacquer in preparing thin sections of soil for microscopic examination. When soils contain large grains of quartz sand, grinding must proceed very carefully in order that the sand grains will not be disturbed. Lacquer is easily scratched when sand grains are dislodged, although it does not soften so quickly as Kollolith when subjected to rapid grinding. It is also difficult to prevent lacquer from forming bubbles when a flat, polished surface of a clod is attached to a glass slide; consequently, some other type of cementing material should be available for this purpose.

Since many different kinds of plastics can be obtained from commercial sources, an attempt was made to obtain as many of these materials as possible

and to determine which of these substances could be recommended for treating soil samples to be used for microscopic study or as a cement to attach prepared samples of soil to glass slides. The materials which were tested are listed in table 1.

Bakelite varnish was not only the best cementing material examined, but it can be used on all types of soil if the ratio of thinner to varnish is varied. Regular thinner, as furnished by the manufacturer, or methyl alcohol containing a small quantity of acetone, as used by Leggette (3), is satisfactory. One part of varnish and two parts of thinner should be used for most soils. If soils are porous the clod can be placed in the varnish for one or two hours, and a

TABLE 1

Source of material tested for value as a cement in the preparation of thin sections of soil for microscopic study

MATERIAL	SOURCE
Bakelite Lacquer No. 3128	Bakelite Corporation, New York, N. Y.
Bakelite Varnish No. 1305	Bakelite Corporation, Chicago, Ill.
Canada Balsam	Local
Clear Lacquer	Local
Durez	General Plastics, Inc., North Tonawanda, N. Y.
Kollolith	Voigt & Hochgesang, Gottingen, Germany
Lauxite Resin	I. F. Laucks, Inc., Seattle, Wash.
Lucite	E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.
Plasto Resin No. 15	Advance Solvents & Chemical Corporation, New York, N. Y.
Resinel No. 3	Glyco Products Company, Brooklyn, N. Y.
Resoglaz	Advance Solvents & Chemical Corporation, New York, N. Y.
Sodium Silicate	Local
Tenite	Tennessee Eastman Corporation, Kingsport, Tenn.
Vinylite Resin "AYAT"	Carbide & Carbon Chemicals Corporation, New York, N. Y.
Vinylseal	Carbide & Carbon Chemicals Corporation, New York, N. Y.

satisfactory infiltration of the solution will occur. In order to obtain thorough penetration in fine-textured soils, the clod should be placed in an aluminum dish and covered with a dilute solution of Bakelite varnish. A partial vacuum should be created by placing the dish containing the soil in a desiccator which can be attached to a vacuum pump. When the vacuum is released, air pressure forces the solution into the soil pores if the clod is submerged in the liquid. Volatile material is removed by placing the treated soil in an oven at 60°C. for about one hour. This method of treatment keeps the varnish from forming bubbles as the solvent escapes. Several millimeters of surface soil should be removed by grinding in order to eliminate excessive concentrations of Bakelite, which may partially fill some of the noncapillary pore spaces.

Bakelite varnish is not satisfactory for use in cementing the polished surface of a clod to a glass slide, because it forms a dark semitransparent material when it hardens. Bakelite lacquer in thin layers is only very slightly colored, and it can be used to cement the surface of polished clods to glass slides so that thin sections can be prepared for microscopic study. Kollolith, Plasto Resin No. 15, and Resinel No. 3 are clear and colorless, but they are not so hard as an ideal cement should be. Soil can be treated with these resins by melting them and placing a small clod in the hot resin for 20 to 30 minutes. Evacuation of the air aids penetration in fine-textured soils when the partial vacuum is released. These resins can be used to cement the treated soil to glass slides if a small quantity of the material on the slide is melted by careful heating. In order to prevent rapid cooling of the resin, the soil should be heated in an oven before it is placed on the slide. This increases the depth of penetration of the resin into the noncapillary pore space in the soil. Other materials which were examined were too soft, too brittle, or too highly colored; would not bind the soil strongly enough to permit grinding; or affected the natural structure of the soil. A pronounced swelling occurred when Lucite was added to soils high in organic matter.

METHOD FOR THE PREPARATION OF THIN SECTIONS OF SOIL FOR MICROSCOPIC STUDY

Small clods of soil one-half to one inch in diameter should be collected from each horizon in a soil profile. If the soil is very loose, samples must be collected in metal containers, air dried, and treated with dilute lacquer solution to hold the soil together for additional treatment. Each sample should be marked so that the vertical or horizontal axis can be easily determined. Place the dry clod in an aluminum dish containing one part of Bakelite varnish diluted with two parts of Bakelite thinner. The clod should be submerged in the varnish. The solution will penetrate porous soils readily, but the air should be removed from fine-textured soils by placing the dish containing the soil and solution in a desiccator which can be attached to a vacuum pump, a mercury manometer being used to determine how much time is required to remove the air from the container. Leave the clod submerged in the Bakelite varnish for 15 minutes after the vacuum is released in order that the solution will penetrate into the fine pores of the soil. Transfer the clod to a small cardboard on which the soil number is marked, and dry in an oven at 60°C. for one hour to remove the solvent. Increase the temperature to 80°C. for one hour and finally to 100°C. for five or six hours. Remove the clod from the oven, cool, and prepare a smooth face by grinding on an emery wheel. A polished surface is prepared by rubbing the smooth face of the clod on No. 1, No. 0, and finally on No. 0000 emery paper. If some of the coarse particles of soil are removed during the grinding process, the sample can be given two treatments with Bakelite varnish or lacquer to develop a stronger bond between the soil particles. The surface of each clod must be well polished and

flat before it is cemented to the glass slide. Remove the dust from the pores in the polished surface of the clod, using air pressure if available. Place about six drops of Bakelite lacquer on a clean glass slide, and press the prepared surface of the specimen into the lacquer. Dry overnight at room temperature. Then dry in an oven at 60°C. for three or four hours and finally at 90°C. until the lacquer is very hard, which usually takes six or seven hours. The lacquer will turn brown if the temperature is higher than 90°C. for any length of time. If a resin is melted on the glass slide as a cementing material, heat the soil in an oven before it is forced into the hot liquid on the glass slide so that a deeper penetration of the resin into the soil pores will occur. Grind the specimen to any desired thickness, as previously described. Clean with air pressure or wash the thin section with 95 per cent ethyl alcohol to remove dust from the pores. The specimen is then ready for microscopic examination.

In many sandy soils, light will pass through the quartz grains, which will appear as pores when examined with a microscope not equipped with special prisms for mineral analysis or in a photograph. If, before the clod is cemented to the glass slide, the surface of glassy minerals is covered with black drawing ink to reduce the quantity of light passing through thin sections, satisfactory pictures can be obtained unless a high magnification is used. Under such conditions, the ink which enters the microcapillaries will shrink when it dries and will form very minute cracks. The black color also interferes with the study of such factors as organic matter distribution and iron cementation. Good photographs can be obtained only when an objective is used which will give a flat field. A 9- by 12-cm. camera with extension bellows equipped with a shutter and a Leitz 24-mm. objective can be used to obtain photographs of low magnification, a projection lantern being used as a source of reflected light. Place an opal glass beneath the slide and allow some light to pass through the slide to delineate the boundary of the noncapillary pore space.

Photographs of thin sections of soil taken at different depths in a Miller clay, an Enterprise very fine sandy loan, and a Portland clay loam profile are shown in plate 1. These soils were magnified 22 diameters when photographed but have been reduced to 4.5 diameters in the illustration. Miller clay has a high percentage of noncapillary pore space in the 6- to 12-inch layer. It is not difficult, therefore, to understand how granulation could occur as a result of the gradual separation of soil masses by root development. Below this depth the soil contains only a few small, round pores. Enterprise very fine sandy loam is porous throughout the entire profile. It contains many large fragments of minerals, chiefly quartz, which appear gray because the polished surface of the specimen was coated with black drawing ink to reduce the quantity of light passing through the minerals as compared with the large pore spaces. Portland clay loam has many large irregular-shaped pores throughout the entire profile. The organic matter content of this soil is high, and the cultivating effect of grass roots as they increase the quantity of macroscopic pore space is evident.

Noncapillary pore space in soils can be estimated by filling, with white casein paste,[1] the pores in a clod which has been hardened and polished. The surface of the soil is photographed by reflected light, and measurements can be made with a planimeter or similar measuring device. This method can be used to measure noncapillary pore space without grinding the soil to a thin section.

SUMMARY

A method has been described in which Bakelite varnish is used to cement soil particles together in order that clods of soil can be ground to thin sections for microscopic examination. Lacquer is satisfactory when soils do not contain grains of sand, which must be cemented firmly or they will be dislodged during the grinding process. Of fifteen different materials studied, only four were suitable for attaching the polished surface of a cemented clod to a glass slide. These substances are Bakelite lacquer, Kollolith, Plasto Resin No. 15, and Resinel No. 3. A method is proposed for the study and estimation of noncapillary pore space by filling the pores appearing in the polished face of a clod with white casein paste.

A study of undisturbed samples of soil under low and high magnification will give accurate information in regard to the character and amount of noncapillary pore space and other physical factors, such as soil granulation, distribution of soil concretions, and the dispersion of soil organic matter, which cannot be obtained by indirect methods of physical analysis.

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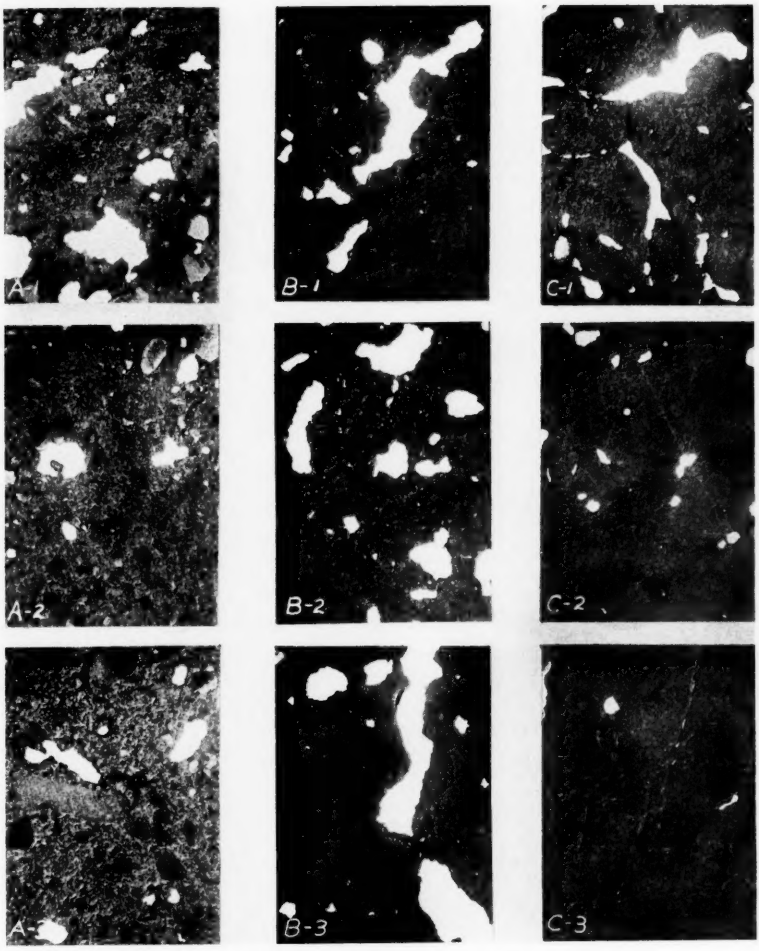
[1] This material, the trade name of which is Texolite, is manufactured by the U. S. Gypsum Company, Chicago, Ill.

PLATE 1

NONCAPILLARY PORE SPACE IN SOIL PROFILES

(A) Enterprise very fine sandy loam, (B) Portland clay loam, (C) Miller clay. A-1, B-1 and C-1 represent a soil depth of 6 to 12 inches; A-2, B-2, and C-2, 18 to 24 inches; and A-3, B-3, and C-3, 30 to 36 inches. Magnification × 4.5.


A-1
B-1
C-1
A-2
B-2
C-2
A-3
B-3
C-3

PHYSICAL CHARACTERISTICS OF SOILS: IV. DENSITY GRADIENTS IN SEDIMENTING COLUMNS AND A CHAINO-HYDROMETER FOR MECHANICAL ANALYSIS OF SOILS

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The use of the hydrometer for the mechanical analysis of soils has been popularized by Bouyoucos (1, 2, 3). The method has been criticized by Keen (4). To overcome some of the objections to the ordinary design, a new type of hydrometer was devised by the author (5). This hydrometer, which has a long thin stem and a short bulb, records density changes in a column 8–10 cm. long and situated 50–60 cm. from the top. The successful working of this hydrometer depends on the fact that the gradient of increasing density in the suspension of a sedimenting column becomes less and less steep as we go from top to bottom. In other words, the average density of a suspension, the length of which is small compared to the total depth of the sedimenting column, may be taken without serious error as the density of its middle point. The practical working of this type of hydrometer revealed two drawbacks, namely, the difficulty of making a long stem stand upright and the liability to breakage.

The hydrometer, though extensively used by road engineers, has never appealed to soil scientists as an instrument of precision which could replace the ordinary pipette method for the routine analysis of soils in the laboratory. The question is of importance, since an eminent soil physicist like Keen, while criticizing Bouyoucos' hydrometer method, has stated (4), "The determination was essentially qualitative since an appreciable density difference must exist in the suspension between top and bottom of the long hydrometer bulb after so short a period as 15 minutes." Further, he has stated, "Hence in physical terms, the hydrometer technique consists in measuring at an arbitrary time the average density of a layer of suspension several centimeters in length, whose density is continually changing both with depth and time. The statement that the method is 'essentially qualitative' is not a matter of personal opinion, but of fact." Keen has laid great stress on his main contention that the hydrometer method is essentially empirical and lacks the precision of theory and experiment associated with the science of pure physics.

One serious drawback in the hydrometer technic as compared to the pipette method lies in the fact that the former does not measure the concentration of particles at one point, but rather gives the average density of a fairly large section of the sedimenting column. This section, moreover, is not situated at a fixed distance from the top but moves with the changing concentrations of

the suspension, which affect the resting point of the hydrometer. The first defect is fundamental and cannot be removed unless a point hydrometer could be produced, or the concentration gradients in sedimenting columns could be examined and the magnitude of errors involved shown to be insignificant. The second defect could be removed by the use of a constant immersion chaino-hydrometer.

The object of this paper is to examine the uniformities of density gradients in the sedimenting columns of natural soils, leading to the development of a chaino-hydrometer of great sensitivity and precision.

DENSITY GRADIENTS IN SEDIMENTING COLUMNS

The basis of the hydrometer method is a single assumption; namely, the average density of any section of a sedimenting column is equal to the density at its middle point. Curiously, this assumption has never been subjected to a rigid test, though the general agreement between the hydrometer and the pipette method has been shown but has been ascribed largely to compensating errors assumed to have been operative in the majority of cases. This leaves the theoretical background unsound, and consequently the element of doubt has persisted.

Complete summation curves of 150 soils were determined by the pipette method. Ten-, fifteen-, and twenty-centimeter sections of the settling column, commencing from a depth 5 cm. from the surface, were considered as representing the lengths of the hydromether bulb. The percentage of particles corresponding to each of several different settling times at the middle point of the section was determined by interpolation from the summation curves and calculated by taking the mean of percentages at the two extremities of this section. It was argued that if these two sets of values agreed among themselves, then the soundness of the assumption that the mean density equals the density at the middle of any section would be established. Further, the limiting length of the hydrometer bulb within which this relation holds, would also be known. For lack of space, the complete table recording these results is not given, but the following brief analysis of the results is equally convincing. The absolute differences between the percentage of particles calculated from the mean density and that determined directly at the middle point, for various sizes, are given in table 1 against the percentage of the total number of soils showing this difference.

It will be seen that in all cases the density gradient for any column below a depth of 5 cm. is symmetrical, and that, therefore, the density at its middle point is equal to the mean of the densities at the extremities. The differences increase as the bulb of the hydrometer is made longer, and only in the case of coarser fractions. For clay estimations the bulb may be as long as 20 cm. without introducing any appreciable error. We are, therefore, justified in taking the average density of any column and assuming it to be equal to the density at its middle point. This conclusion must set at rest any misgivings

regarding the use of the hydrometer for the mechanical analysis of soils and the acceptance of such results as truly representing the size distribution of particles.

It must be admitted that there is no theoretical reason why the density gradients of sections of sedimenting columns of soils should show such a uniformity, but there is also none why they should not, and therefore we must accept the facts as they are.

The results heretofore considered refer to soils completely dispersed by the Na_2CO_3-NaOH method (6), but the relation is equally valid when the soils are subjected to no preliminary treatment for dispersion and are simply left in

TABLE 1

Comparison of percentages of particles calculated from the mean densities of sections of settling columns with those from densities at their middle points

Limiting Sizes of Particles.....*mm.*	0.05			0.02			0.01			0.005			0.002		
Length of hydrometer bulb.....*cm.*	10	15*	20*	10	15	20	10	15	20	10	15	20	10	15	20
Difference between percentages found by two methods	Percentage of soils														
10	0	2.1	4.2	0	0	0	0	0	0	0	0	0.7	0	0	0
9	0	2.1	2.1	0	0	0	0	0	0	0	0	0	0	0	0
8	0.7	0	2.1	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	4.2	0	0.7	2.0	0	0	0	0	0	0	0	0	0
6	0	2.1	2.1	0	0	0.7	0	0	2.7	0	0	1.3	0	0	0
5	0.7	4.2	6.3	0	2.6	3.3	0	0.7	1.3	0	0.7	1.3	0	0	0
4	0.7	6.3	2.1	0.7	2.1	6.7	0	0.7	4.0	0	2.0	2.7	0	0	0
3	2.0	10.4	10.4	3.3	8.7	17.3	0.7	3.3	7.3	0	3.3	3.3	0	0	2.1
2	12.6	16.7	22.9	14.0	24.6	22.7	4.0	17.3	24.7	4.6	9.3	16.7	0	0	0
1	39.3	31.3	25.0	36.6	36.7	30.7	22.0	44.0	34.0	21.3	30.7	30.7	0	0	14.6
0.5 and less	44.0	25.0	18.7	45.4	24.6	16.7	73.3	34.0	26.0	74.1	54.0	44.0	100.0	100.0	83.3

* 48 samples only, for each of these lengths, were analyzed for this size.

contact with water. Sixty undispersed soils were examined, and their summation percentage curves showed that the difference between the density at the middle point and the mean density for a 10-cm. section in no case exceeded 3.6 per cent reckoned in terms of absolute percentage of any fraction, and only two soils showed a difference of this order, and that too in the case of coarser fractions only; three soils showed a difference of 3 per cent, again in coarser fraction only; and all the rest gave a difference less than 2 per cent, the majority giving less than 1 per cent. This is a remarkable fact and one that enhances the value of the hydrometer method for determining the state of aggregation of compound particles in soils (7).

CHAINO-HYDROMETER

It is well known that measurements of weight can be made with far greater accuracy than can those of volume. It is for this reason that the analytical balance has never been surpassed for measuring densities of liquids and that the use of the hydrometer has been accepted as a rough-and-ready method only on account of the great saving in time. The chaino-hydrometer resulted from

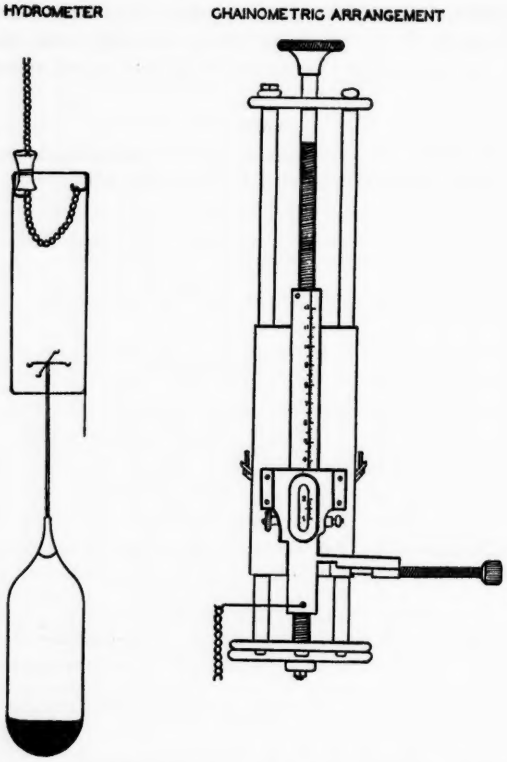

FIG. 1. THE CHAINO-HYDROMETER AND THE CHAINOMETRIC ARRANGEMENT

our attempt to combine the rapidity of the hydrometer and the precision of the analytical balance. It has proved to be an extremely sensitive instrument for measuring densities of liquids, especially soil suspensions.

The hydrometer, the principle of which is embodied in the well-known Nicholson's hydrometer, is shown in figure 1. It is made of brass and is chromium plated. The lower part of the stem of the hydrometer is shaped from a steel knitting needle, and the rectangular loop at the top is made of stout steel wire and carries a fine needle point. This needle point indicates

the limit to which the hydrometer is to be immersed. With this device, the immersion of the hydrometer can be reproduced to the fraction of a millimeter. In one corner of the loop is fixed an aluminum tube; and in the other, a hook. A fine chain 25 cm. long and weighing 0.5 gm. passes through the tube and, forming a loop, ends on the hook. The chain hangs from a hook which can be raised or lowered by a thumb screw working a vernier scale. A movement of 1.6 cm. on the scale corresponds to a change of 10 mgm. in the weight of the hydrometer. A total weight of 100 mgm. could be added by the chainometric arrangement. Larger weights are added directly on the special hooks in the center of the loop in continuation of the stem.

For uniformity of results, the chain must have a fixed initial loop when the vernier registers zero. This is accomplished by the help of another screw, shown at the top, which can move the whole chainometric arrangement. This is worked until a fixed number of chain rings or a fine mark on the chain has passed across a pointer. From this point onward the vernier scale is manipulated until the needle touches the surface of the liquid, when the hydrometer registers the true density of the suspension. It is important that the initial loop of the chain be made immediately before a reading is taken, and the final reading should always include a loop which is bigger than the initial loop.

We made some hydrometers from the bulbs of 100-cc. and 50-cc. pipettes by sealing both ends and attaching rectangular steel stems with sealing wax. If such a hydrometer breaks, a new one can be constructed, ready for use, within a few hours. The brass ones are, however, preferable. The glass hydrmoeters are weighted with mercury and the brass ones with lead shots.

TEMPERATURE CORRECTION

For a sensitive hydrometer, temperature correction is absolutely essential. This is calculated by weighing the hydrometer and taking into consideration the fact that the whole of that weight is lost when the hydrometer floats freely in water. The volume of the immersed portion of the hydrometer is obtained from the volume of an equal mass of water at that temperature. Knowing the coefficient of expansion of water with temperature, we can construct a table which will give at a glance the weight of the hydrometer at various temperatures. Thus if W is the weight of the hydrometer and W_t the additional weight added to bring it to the constant immersion point at temperature t we have:

$$W + W_t = V \times d_t$$

where V is the volume of the immersed portion of the hydrometer and d_t the density of water at t°C. Thus, after one reading is taken at any temperature, the values of the additional weight at other temperatures are calculated from the known values of the density of water at these temperatures. In this relation we have assumed that V, the volume of the hydrometer, remains constant. Since the hydrometer is extremely sensitive, the results are affected

by the expansion or contraction of the material forming the bulb, and a positive or negative correction has to be applied when constructing the temperature correction chart.

If V_t is the volume of the hydrometer at temperature t, then its volume at any other temperature t' is given by the formula:

$$V_{t'} = V_t \{1 + \gamma (t' - t)\}$$

where γ is the coefficient of cubical expansion of the material forming the hydrometer. Thus the complete temperature correction chart can be constructed from the formula:

$$W + W_t = V_t \times d_t.$$

Thus one single weighing to find W and one single reading at any temperature t to find W_t suffices to construct the complete temperature correction chart. Actually the values of W_t are taken at three or four temperatures to check the calculated and found values. The values of W_t need not be calculated for fractions of a degree; this can be done for whole numbers, and the results plotted on an open scale from which the intermediate values for fractions of a degree are interpolated when preparing the temperature correction chart. Evidently the temperature correction chart gives the change in the density of water in terms of W_t. We also know that a change of density of 0.001 from that of water would produce a change of $0.001 \times V$ gm. in w where V is the volume of the hydrometer. Thus if the volume of the hydrometer is 100 cc. and if with the help of the chainometric arrangement w could be determined correct to a milligram, the densities could be found correct to the fifth decimal place. Actually an accuracy of this order is not necessary for the mechanical analysis of soils. The excess of density of 1 per cent soil suspension over water at the same temperature is 0.006269, assuming a density of 2.68 for the soil particles. This will produce a change of $0.006269 \times V$ gm. in the weight of the hydrometer. Thus the percentage of particles of any fraction in a 1 per cent suspension of soil is equal to

$$\frac{(w' - w) \times 100}{V \times 0.006269}$$

where w' is the additional weight at the constant immersion point and w is the weight of water at the same temperature (given in the temperature correction chart). As V is constant, the multiplying factor is calculated only once. Strictly speaking, V changes slightly with temperature, but this change is negligible for this purpose and need not be considered. It will be seen that if V is made equal to 159.5 cc., the multiplying factor becomes equal to 100, and $(w' - w)$ in milligrams gives directly the percentage of any fraction for a 1 per cent suspension. Alternately, if the concentration of the suspension is made equal to $\frac{159.5}{V}$, the factor will be 100.

A hydrometer may be tested occasionally at room temperature in distilled water, and if any change in the weight (w) is observed, a positive or negative correction is applied to all the values in the temperature calibration chart.

It is worthy of note that the hydrometer gives the density of the suspension, whereas the pipette method gives directly the percentage of suspended matter by weight. Agreement between the two methods can be expected only if the density of different soils is the same. We have assumed a density of 2.68 for all soils. This figure was obtained by actual measurements of density in a number of mineral soils; the average density was found to be 2.68, the lowest value being 2.64 and the highest 2.71. It might be mentioned that a difference of 0.04 in the actual density of the soil from the mean value will produce a difference of 1 per cent in the weight of the fraction. Since most of the mineral soils approximate the mean value, the difference between the hydrometer and the pipette method is not likely to be serious on this account.

TABLE 2

Comparison between the hydrometer and the pipette method for determining percentages of fractions

Limiting Sizes of Particles.....mm.	0.02	0.01	0.005	0.002
Difference between percentages found by two methods	Number of soils			
4	3	1	1	0
3	3	2	2	0
2	10	7	9	4
1	13	19	22	12
0.5 and less	32	32	27	45

COMPARISON WITH THE PIPETTE METHOD

An important consideration that arises in the use of a hydrometer is the disturbance likely to be caused by the insertion of a large body in a settling column. How far this may affect the accuracy of results can be determined only by a direct comparison between the hydrometer and the pipette method. Sixty-one soils were analyzed by the two methods. The results are grouped in table 2. The absolute differences in the percentages of various fractions are given against the number of soils showing a difference of that order. The agreement is very good, in view of the fact that even in the pipette method a difference of the order of 2 in the percentage of any fraction is within the experimental error of the instrument.

REPRODUCIBILITY OF RESULTS

Reproducibility of results by the hydrometer method was compared with that by the pipette method. Fourteen soils were analyzed five times by both methods. The number of replicates for finer fractions was reduced to four, as accuracy is greater when the time of settling is long. To eliminate sampling

TABLE 3

Reproducibility of results with the hydrometer and the pipette method

Limiting Size of Particles .....mm.		0.02			0.01			0.005		
Soil Number		Hydrometer 1	Hydrometer 2	Pipette	Hydrometer 1	Hydrometer 2	Pipette	Hydrometer 1	Hydrometer 2	Pipette
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
P.C. 13	Max.	79.21	80.99	80.25	70.43	72.45	72.30	64.92	64.20	66.20
	Min.	77.97	79.81	78.15	69.54	70.39	69.60	63.58	63.02	63.10
	Av.	78.31	80.16	78.91	69.95	71.33	70.50	64.33	63.69	64.25
P.C. 123	Max.	95.15	95.71	96.90	93.51	92.18	92.20	90.83	91.00	91.65
	Min.	94.40	95.12	92.85	92.17	91.30	89.90	89.94	88.35	89.70
	Av.	94.76	95.48	95.08	92.71	92.00	91.60	90.40	89.82	90.59
P.C. 166	Max.	77.73	75.39	74.30	70.73	68.91	69.25	62.54	60.67	59.15
	Min.	77.43	73.04	72.40	69.24	67.14	65.80	61.79	57.40	56.75
	Av.	77.58	74.10	73.69	69.86	68.09	67.00	62.17	58.82	58.11
R.M. 23	Max.	72.07	72.15	73.85	62.98	64.20	63.80	49.29	49.48	52.75
	Min.	71.62	71.56	71.00	61.64	62.14	61.05	48.84	47.12	48.35
	Av.	71.86	71.80	72.80	62.30	63.38	62.43	49.07	48.45	50.44
R.M. 24	Max.	87.26	88.06	88.20	77.58	77.75	80.17	62.69	62.43	64.15
	Min.	86.36	87.17	86.90	76.24	76.57	77.10	61.05	60.37	61.30
	Av.	86.84	87.59	87.54	76.89	77.16	78.17	61.87	61.48	62.74
R.M. 80	Max.	64.77	65.97	65.30	50.18	53.01	51.15	37.23	39.46	38.90
	Min.	62.24	63.32	64.50	48.84	48.59	48.95	36.18	37.11	36.95
	Av.	63.25	64.56	64.96	49.41	50.60	50.00	36.74	37.89	37.91
R.M. 88	Max.	46.31	48.89	48.25	37.23	38.29	37.60	27.84	28.27	29.00
	Min.	45.85	47.12	47.00	35.14	35.34	35.30	27.10	25.92	27.00
	Av.	46.10	47.59	47.61	36.13	36.64	36.67	27.40	26.73	27.80
R.M. 94	Max.	48.24	48.59	47.80	36.33	39.17	37.00	12.95	12.66	15.60
	Min.	46.61	47.12	46.25	34.21	37.40	35.70	12.21	11.78	13.65
	Av.	47.74	47.71	46.97	35.40	38.17	36.36	12.55	12.22	14.64
R.M. 96	Max.	80.85	83.93	84.05	67.15	69.21	66.25	49.43	50.01	52.10
	Min.	79.96	81.58	81.75	65.52	66.85	63.90	48.84	49.48	48.70
	Av.	80.50	82.81	82.86	66.35	67.97	65.32	49.17	49.63	50.44
R.M. 99	Max.	40.20	41.52	42.45	23.08	23.85	26.40	9.08	9.42	10.20
	Min.	38.71	40.05	40.60	21.59	22.97	23.00	8.19	8.25	8.25
	Av.	39.54	40.58	41.32	22.19	23.44	24.04	8.71	8.98	9.28
R.M. 100	Max.	11.17	12.66	12.45	10.13	11.19	10.60	9.68	7.66	10.05
	Min.	10.42	11.19	11.00	8.93	10.01	9.25	8.79	7.36	9.00
	Av.	10.75	11.72	11.62	9.53	10.60	9.76	9.31	7.51	9.76

TABLE 3—*Concluded*

Limiting Size of Particles..... *mm.*		0.02			0.01			0.005		
Soil Number		Hydrometer 1	Hydrometer 2	Pipette	Hydrometer 1	Hydrometer 2	Pipette	Hydrometer 1	Hydrometer 2	Pipette
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
R.M. 104	Max.	40.20	41.23	41.40	30.23	30.34	30.90	21.89	21.50	22.80
	Min.	39.76	40.35	38.85	28.14	29.16	28.20	20.85	19.73	20.20
	Av.	39.99	40.76	39.78	29.04	29.75	29.38	21.37	20.76	21.93
R.M. 106	Max.	53.60	54.78	55.70	34.25	36.81	36.50	20.25	21.50	21.60
	Min.	52.12	53.01	54.00	32.76	35.34	35.90	19.21	20.32	20.15
	Av.	52.95	53.83	54.56	33.79	35.93	36.17	19.62	20.69	20.98
R.M. 115	Max.	29.78	30.33	30.75	22.34	23.27	21.35	15.63	15.31	15.60
	Min.	28.74	28.27	29.85	21.14	21.50	20.65	14.74	14.14	13.70
	Av.	29.39	29.45	30.28	21.89	22.21	21.01	15.15	14.65	14.69

Hydrometer 1: Length of bulb = 12.4 cm.; volume = 106.85 cc.
Hydrometer 2: Length of bulb = 10.8 cm.; volume = 54.00 cc.

errors, the same suspension was used for both methods. The results, given in table 3, show that the reproducibility of results is actually better with the hydrometer method than with the pipette method. These results also emphasize the close agreement between the hydrometer and the pipette method.

It might be pointed out that there is nothing arbitrary or empirical in the use of the chaino-hydrometer. The settling times of the particles of various sizes are calculated from Stokes' law to a depth corresponding to the middle point of the hydrometer bulb. If the bulb is not symmetrical about the middle point, then the mean density point corresponds to the center of gravity of the surface of the bulb and is calculated as follows:

The bulb is laid on graph paper with its axis horizontal, and its outline is then traced with a pencil held vertically. The resulting closed curve (fig. 2 a) forms a vertical projection of the surface of the bulb, and as the latter can be held to be generated by the revolution of either half of the curve about the line into which the axis projects, the following relation holds between the area A_1 of either half and the surface S of the bulb:

$$S = 2\pi \cdot A_1 \qquad (1)$$

To find the coordinate of the center of gravity of the *surface* of the bulb, another curve (fig. 2 b) is drawn in which the coordinate is the product of the abscissa (x) of any point, as shown in fig. 2 a, and the corresponding ordinate (y). The area of this curve equals A_2, where:

$$A_2 = \int_0^a xy \cdot dx \qquad (2),$$

a being the length of the axis.

The abscissa of the center of gravity of the *surface* of the bulb then, according to the usual definition of the center of gravity, is given by

$$S \cdot \bar{x} = \int_0^a x \cdot 2\pi y \cdot dx \tag{3}$$

or

$$\bar{x} = \frac{2\pi \int_0^a xy \cdot dx}{S}$$

$$= \frac{2\pi A_2}{2\pi A_1} \qquad \text{from } (1) \text{ and } (2)$$

$$= A_2/A_1.$$

The ordinate of the center of gravity will be zero, since the bulb is symmetrical about the axis.

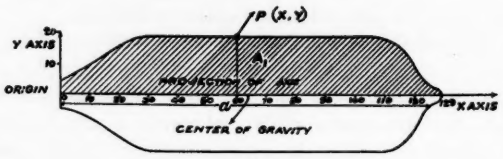

FIG. 2a

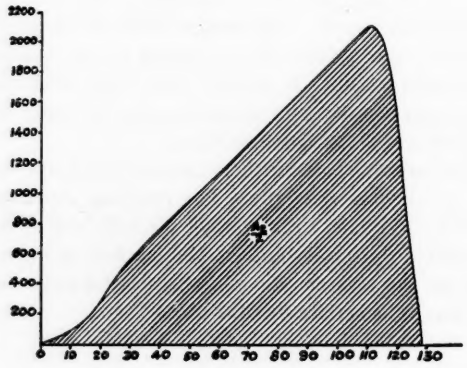

FIG. 2b

FIG. 2. METHOD OF CALCULATING THE MEAN DENSITY POINT OF THE HYDROMETER BULB

As the vertical scale of the ordinate in b is compressed to half, the area enclosed by the cube is one-half A_2.

The hydrometer bulb, of course, can be made symmetrical, but we actually used hydrometers in which the bulbs were made from 100-cc. and 50-cc. pipettes. The mean density points were calculated in these cases, and pipet-

ting for comparison was done at these points. The object of introducing this discussion is to emphasize the fact that the *chaino-hydrometer does not lack the precision of theory and experiment associated with the science of pure physics*, a criticism offered by Keen for the hydrometer method in general. The hydrometer is lowered in the suspension just before the calculated settling time for the required size of particles to the depth corresponding to the middle point or the center of gravity of the bulb of the hydrometer, just as the pipette is lowered, in the pipette method. The disturbance caused is negligible, being no more than that in the pipette method. The hydrometer is not recommended for continuous readings by keeping it in the suspension and taking observations at definite intervals of time. Apart from the errors introduced by the actual settling of the particles on the bulb of the hydrometer, such a procedure would require a separate hydrometer for each suspension, and thus a number of soils could not be examined simultaneously without adding to the cost of equipment. The object of the chaino-hydrometer is to replace the pipette method with a technic that is simpler, quicker, and more accurate. We have mounted the chainometric arrangement on a robust stand that can slide between rails. The 1- or 2-liter cylinders containing the soil suspensions are arranged in a row, and the chainometric arrangement is brought behind each cylinder one after the other at the appropriate time.

After the rigid and exhaustive comparison of the two methods, the pipette method has been replaced by the hydrometer in this laboratory. We find that complete mechanical analysis of the soil can be made in the time formerly required for the determination of the clay fraction only by the pipette method. The saving effected is not only in time but in the cost of equipment for dishes and heaters, which is considerable.

SUMMARY

From an examination of the density gradients in suspensions of 150 soils, it is concluded that any section 10–12 cm. in length and situated 5 cm. below the surface of the settling column has an average density which is equal to the density at its middle point. This fact makes it possible to measure the density and hence the percentage of particles at any point in a settling column with a hydrometer. For this purpose, a chaino-hydrometer of high sensitivity with a chainometric attachment has been devised and its use for the mechanical analysis of soils discussed.

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